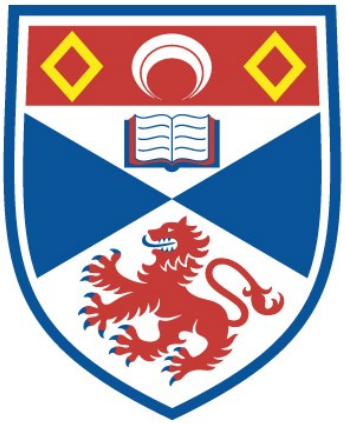
A COMPARITIVE STUDY OF THE VENTRICLES AND THE BULBUS CORDIS OF THE VERTEBRATE HEART

Hrishikesh Das Biswas

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1957

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A THESIS

presented for the Degree of

Doctor of Philosophy

of

The University of St. Andrews

by

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CERTIFICATE

I certify that Hrishikesh Das Biswas has spent nine terms at Research Work under me in the Department of Anatomy, St. Salvator's College, that he has fulfilled the conditions of the Ordinance No.16 (St.Andrews) and that he is qualified to submit the accompanying Thesis in application for the Degree of Ph.D.

M.D., F.R.C.S.Ed., F.R.S.E.

Professor of Anatomy,

St. Salvator's College,
University of St.Andrews.

mc
1990.

DECLARATION

I hereby declare that the following Thesis is based on macroscopic and microscopic specimens prepared by me, that the Thesis is my own composition, and that it has not previously been presented for a Higher Degree.

The Research was carried out in the Department of Anatomy, St. Salvator's College, University of St.Andrews.

CAREER

I matriculated in the Patna University (India) and followed a course leading to graduation in 1948. From 1949 to August 1955, I was a Demonstrator of Anatomy in the Assam Medical College, India.

In October 1955 I was admitted as a Research Student in St. Salvator's College, University of St.Andrews, and there commenced the work in the Anatomy Department of that College, which forms the subject of this Thesis.

ACKNOWLEDGEMENTS

I am deeply indebted to Professor R. Walmsley, M.D., F.R.C.S.Ed., F.R.S.E., for his constant guidance, criticism and encouragement throughout this work. I also wish to express my deep sense of gratitude to Dr. J.H. Mulligan for his advice and assistance especially in nerve staining. My thanks are also due to Mr. J. Brown and his assistants for preparing the photomicrographs and to Miss Thompson for typing the Thesis.

CONTENTS

LIST OF ABBREVIATIONS USED IN TEXT FIGURES AND PLATES

Atrium	Atr.
Atrial wall	Atr.W.
Atrioventricular funnel	A.V.F.
Atrioventricular sulcus	A.V.S.
Atrioventricular valve	A.V.V.
Atrioventricular opening	A.V.O.
Atrioventricular bundle	A.V.B.
Aorta	Ao.
Aortic arch, right	Ao.arch Rt
Aortic arch, left	Ao.arch Lt
Aortic root	Ao.R.
Anterior papillary muscle	A.P.M.
Anterior horn of the vortex	A.H.V.
Anterior crus of the Infundibuloventricular crest	A.cr.I.V.C.
Bulbus arteriosus	B.A.
Bulbus cordis	B.C.
Blood sinus	Bl.Sin.
Body of the Infundibulo--ventricular crest	B.I.V.C.
Capillary	Cap.
Circular layer	Cir.L.
Circular layer of the left ventricle	Cir.L.Lt.V.
Conus tendon	C.T.

Cavity of the right ventricle	Cav.Rt.V.
Deep bulbospiral layer	D.B.S.
Deep sinospiral layer	D.S.S.
Dorsal wall	D.W.
Dorsal interventricular groove	D.I.V.G.
Epicardium	Epi.
Ganglion	Gang.
Inner layer	I.L.
Infundibulum	Infund.
Left atrium	Lt.Atr.
Left aorta	Lt.Ao.
Left ventricle	Lt.Vent.
Longitudinal layer	Long.L.
Left atrioventricular opening	Lt.A.V.O.
Left annulus fibrosus	Lt.An.Fib.
Longitudinal layer of the ventricular septum	L.V.S.
Myocardium	Myo.
Nerve bundle	N.B.
Nerve fibre	N.F.
Nerve cell	N.C.
Pulmonary artery	P.A.
Posterior horn of the vortex	P.H.V.
Posterior papillary muscle	P.P.M.
Posterior crus of the Infundibuloventricular crest	P.cr.I.V.C.

Right atrium	Rt.Atr.
Right aorta	Rt.Ao.
Right ventricle	Rt.Vent.
Right atrioventricular opening	Rt.A.V.O.
Right annulus fibrosus	Rt.An.Fib.
Right atrioventricular valve	Rt.A.V.V.
Spiral septum	Sp.Sept.
Satellite cells	S.C.
Strands of terminal nerve network	T.Nerve N.
Superficial bulbospiral layer	S.B.S.
Superficial sinospiral layer	S.S.S.
Terminal fibres of the deep sinospiral layer	D.S.S.Tr.Fr.
Trigonum fibrosum dextrum	T.F.D.
Trigonum fibrosum sinistrum	T.F.S.
Tunica adventitia	Tun.Ad.
Tunica media	Tun.Md.
Ventricle	Vent.
Ventricular trabeculae	Vent.Tr.
Ventral aorta	V.Ao.
Ventral wall	V.W.
Ventral interventricular groove	V.I.V.G.
Ventricular septum	Vent.Sept.

INTRODUCTION

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Although there is a large measure of agreement about various aspects of cardiac anatomy, the problem of the architecture of the ventricular musculature still remains a matter of discussion. This muscle problem is an old one as in the middle of the seventeenth century Stenson (1630) and Lower (1669) first tried to unravel the ventricular musculature. During the nineteenth century there was a considerable controversy regarding the architecture of the ventricles, but the work of Pettigrew (1864) and later that of MacCallum (1900), Mall (1911) and Robb and Robb (1938, 1942) greatly elucidated this problem. There are, however, a number of disagreements in the findings of these investigators but they are unanimous in their conclusions that the ventricles are composed of several separate layers of muscle. Recently Lev and Simkins (1949) have denied such a concept of muscle layers in the ventricular wall and this has once again stimulated interest in the subject.

The intrinsic nerves of the ventricles have likewise been frequently investigated. Most workers on this subject agree about certain aspects of cardiac innervation including the dual source of supply, the general plexiform arrangement of the bigger nerves but there is sharp divergence of opinion about the topography of the intrinsic cardiac nerve cells and the terminal arrangement of the cardiac nerves. There are repeated instances in the literature, of the different workers, examining the hearts of the same animals but employing different methods, reporting findings which bear little

resemblance to each other. It is probable that the different interpretations might be due to the different techniques used and more especially so when the capricious nature of the staining of nerves is remembered. It appears to the writer that this vexed problem of autonomic end-formation will only be definitely solved when a stain is found which allows all nerves and nerves alone in a viscus to be identified.

However, at present, there appears to be two groups of thought about the ventricular innervation. There are those on the one hand, who believe that in the ventricles, the cardiac nerves divide into finer and finer branches, each of which ultimately terminates in an organised ending which lie on the surface or inside the protoplasm of whatever cell the nerve may be supplying, no matter whether it is a muscle fibre or a fat cell. (Berkley, 1894; Morison, 1926; Woollard, 1926; Nonides, 1939, 1941, 1943; King, 1939; Stotler and McMahon, 1947; Toheng, 1951 and Davies et al, 1952). On the other hand there are those who are of the opinion that the ultimate innervation is through a peripheral nervous network with a number of nuclei lying in its meshes (Stöhr, 1932, 1935; Boeke, 1932, 1949; Reiser, 1934; Seto, 1936; Akkeringa, 1949; Field, 1951; Mitchell, 1953; Meyling, 1953; Sato, 1954 and Holmes, 1956, 1957). The mode of formation of the network and the nature of the cells found in it are also much debated. Some workers think that the cells are either connective tissue cells or Schwann cells. (Nonides, 1937, 1939;

Seto, 1936; Weddell and Zander, 1951 and Sato, 1954). Others hold the view that these are small nerve cells, an idea which was first postulated by Cajal who called them "interstitial cells"; this view is accepted by Leeuwe (1937); Boeke (1949); Mayling (1953); Mitchell (1956) and Honjin (1956).

Search of literature reveals that most of the investigations, both about ventricular muscle and its innervation, have been carried out on mammals and rarely on other vertebrates. The present investigation embraces a wide field as an attempt has been made to study the ventricular architecture and its innervation in the different classes of vertebrates and it is hoped that a continuation of this work may ultimately help to throw light on the same problem in the mammals.

The thesis is devoted in the main to a review of the outgoing chambers (ventricles and bulbus cordis, if present) of the hearts in fish, amphibia, reptiles, birds and mammals. The muscular architecture and innervation are treated separately in two parts and at the end of each part there is a discussion on the subject. The discussion is based largely on the relevant literature but also, though in much lesser measure, on my personal findings. At the beginning of each part a short review of the previous work is given.

The ventricular architecture differs considerably in various vertebrates and therefore, observations on it are recorded separately for each class of animal. On the other hand the basic pattern of

innervation is found to be similar in the various animals which I have examined and hence to avoid repetition of the same findings, observations on nerve supply in all animals are given in one chapter.

It was my original intention to include in this study a consideration of the vascular pattern of the ventricles and bulbus cordis. My investigations of the musculature and nerves of these heart chambers have occupied my complete period of study, but it is my intention to continue work on the heart, including its vascularization, on my return to Assam.

METHODS AND MATERIALS

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Hearts of the following adult animals were used; the number of hearts of each species examined is noted in brackets.

1. Mammals:- (a) Ox, (Bos taurus) (6).
   (b) Sheep, (Ovis aries) (11).
   (c) Pig, (Sus domesticus) (2).
   (d) Dog, (Canis familiaris) (2).
   (e) Albino rat, (Mus norvegicus albinus) (8).
2. Aves:- (a) Domestic fowl, (Gallus domesticus) (12).
3. Reptiles:- (a) Green lizard, (Lacerta viridis) (8).
   (b) Grass snake, (Natrix natrix) (6).
4. Amphibia:- (a) Frog, (Rana temporaria) (8).
5. Pisces:- (a) Cod, (Gadus morrhua) (15).
   (b) Haddock, (Gadus aeglefinus) (10).

Among the mammalian hearts those of the ox, sheep and pig were used for the study of the ventricular muscular architecture whereas in those of the dog and rat the innervation only was investigated. In all the other classes of animals both the musculature and nerves were studied.

The muscular architecture of the ventricles was studied by the use of the following methods.

The mammalian and avian fresh hearts were first stuffed with oatmeal to prevent contraction. Then in slightly acidulated water, mammalian hearts were boiled for one to three hours according to the

size of the heart and birds' hearts were simmered for 15 - 20 minutes. It was found to be detrimental to boil the hearts for too long a time as the muscle fibres become very soft and fragile. After boiling, the epicardium, fat, nerves, blood vessels etc. were removed and the atria detached from most of the ventricles. The ventricles were then preserved in 90% alcohol to harden and this change facilitates the separation of the muscle layers; it was found that the large hearts required 3 - 4 weeks immersion in alcohol but the smaller hearts needed only 1 - 2 weeks. At the end of the necessary periods, hearts were dissected with forceps and the blunt end of a scalpel. The reptilian, frog and fish hearts were treated in 90% alcohol for a week to 10 days and then dissected under a binocular microscope. In the dissection of these smaller hearts, it is convenient to use small sharpened wooden sticks like match sticks and it was found that after using them for a while the sharpened end becomes slightly blunt and also serrated which helps in the separation of the muscle fibres. Two of the smaller hearts of each species were also stained with 0.05% Benzoquinone after proper dehydration and cleared in oil of Wintergreen and then the muscle fibres were traced under a binocular microscope. A few of these small hearts were fixed in 10% neutral formol saline and carried through to paraffin and then serial sections, 10 μ thick, were cut in both the sagittal and transverse planes; these were stained with haematoxylin and eosin. A few of the sagittal sections of the fish hearts were also stained with Krajian elastic tissue stain.

For the neurological study, Gros-Schultze technique for frozen sections and Bodian's (1937) activated Protargol method for paraffin sections were used. All animals except fish were killed by gas and immediately fixed in 20% neutral formalin for Gros-Schultze technique and in 10% formol saline or in Bodian's fluid (formol 5 cc., glacial acetic acid 5 cc. and 80% ethyl alcohol 90 cc.) for Bodian's method. Small hearts were used entire for sectioning but in the larger hearts as of the dog and domestic fowl, blocks of tissues were sectioned. Sections of 20 μ thickness were cut for Bodian's method and for Gros-Schultze method they were 40 μ - 60 μ. Three rat hearts (one adult, one 37 days and one 17 days old) were fixed and impregnated in bulk by Nonidez's (1939) method and 10 μ sections were cut and examined.

The writer had not much success with methylene blue technique as the dye diffused out to other tissues and the whole ventricles (even of small animals) did not become transparent enough to allow finer nerves to be seen.

The writer further wants to stress the need for rapid and adequate fixation immediately after the death of the animal as post-mortem changes diminish the silver affinity and consequently it becomes difficult to stain the nervous elements.

PART I

MUSCULAR ARCHITECTURE OF THE BULBUS CORDIS AND THE VENTRICLES OF THE VERTEBRATE HEART

SHORT REVIEW OF THE LITERATURE ON THE VENTRICULAR MUSCULAR ARCHITECTURE

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Stenson (1630) investigated the musculature of the mammalian ventricles and noted that the superficial fibres descend to the apex where they become deep and then ascend in the deepest part of the myocardium. He also noted that the apex is star-shaped and closed by only serous membranes. Lower (1669) noted the formation of loops with both ends attached to the fibrous rings at the base of the ventricles. Winslow (1711) described the ventricles as "two (muscular) bags contained in a third". Senac (1740) first recognised the spiral arrangement of the ventricular muscle fibres. Wolff (1780) claimed that there is also a band of superficial longitudinal muscle fibres, which was additional to anything described up to that time.

The next important observation was made by Gerdy (1823) who first described the "figure of eight" course of the ventricular muscle fibres. He also noted the existence of the interventricular muscle layer which forms the septal wall of the right ventricle. This layer extends caudally towards the inner surface of the diaphragmatic wall of the left ventricle and finally terminates in the base of this ventricle. Weber (1831) noted the circular arrangement of some of the muscle fibres of the left ventricle.

In 1864, Pettigrew after dissecting a large number of hearts, recorded the gradual change in the obliquity of the ventricular muscle fibres in the mammalian and avian hearts and on that basis he divided the ventricular wall into seven layers, three external, one middle and

three internal. The internal layers are described as being continuous at the apex with the external layers. Pettigrew numbered the layers from the epicardial surface to the endocardial as 1 to 7, and showed that, at the apex, layer 1 was continuous with layer 7, 2 with 6, and 3 with 5. Ehrel (1891) first described the conus tendon and also stated that the circular layer (which he described as cylindrical) of the left ventricle is not attached to the fibrous ring at the ventricular base.

MacCallum (1900) unrolled the ventricles of pig into a scroll and demonstrated that when the superficial layer is removed, the deep muscle layers are seen to wind round the walls of both ventricles. Mall (1911) amplified the work of MacCallum and tried to put the subject of the arrangement of the ventricular muscle on a comprehensive basis. He classified the muscle layers according to their origin as "bulbospiral" and "sinospiral" groups. In 1912 he studied the development of the ventricular muscle layers.

Shaner in 1923, dissected the hearts of fish, amphibia, reptiles and birds which formed the basis for a short comparative study of the ventricular muscle. In 1924 he studied the ventricular architecture of the alligator's heart and noted its close resemblance to that of the higher vertebrates and in 1928 he investigated the development of the muscular architecture of the ventricles of the pig's heart. As far as I am aware, Pettigrew and Shaner are the only two workers who have published accounts of the ventricular musculature of animals other than mammals.

Flett (1928) investigated the muscular architecture of the human heart and made an effort to correlate function with structure. Walmsley (1929) suggested that the spiral arrangement of the ventricular muscle fibres is the result of torsion of the ventricular loop in course of development. Walmsley (1938) in his observations on the vascular system of female finback states that each ventricular system of myocardium is complete in itself and consists of a central layer of circular fibres and superficial and deep layers of oblique and longitudinal fibres.

Robb and Robb (1938 and 1942) after studying the mammalian ventricles observed that they are composed of several separate layers, each of which subserves a separate function and furthermore has its own blood supply. Davies and Francis (1941) studied the heart of salamander with special reference to its conducting system and found that no specialised conducting system is present in this animal. The muscle of all the chambers of the heart forms a continuum and shows the same histological features. They also observed that the muscle fibres in all the chambers, are arranged in a thin superficial circular layer and in a "basket work" fashion in the rest of the myocardium but in the junctional areas they are arranged circularly.

Lev and Simkins (1949) disagreed with this concept of there being a number of muscle layers in the ventricular wall. They are of the opinion that the ventricular myocardium consists of one muscular syncytium, made up of fibres arranged in fasciculi of various sizes. However, they have recognised three depths of fasciculi, epicardial, middle and

endocardial, without connotation that any connective tissue cleavage planes separate the syncytium into laminae. Brandt (1953) studied the structure and functions of the Infundibulo-ventricular crest of the human heart and described it as a separate muscle with its origin and insertion.

OBSERVATIONS

OBSERVATIONS

General Remarks:-

Before describing the muscular architectural pattern of the ventricles of various vertebrate hearts, it is considered worth-while to emphasise that the myocardium consists of one muscular syncytium and that the muscle layers which are to be described are, therefore, not really to be regarded as discrete layers with complete connective tissue planes separating them as is found between two somatic muscles. The layers have always been found to be in constant communication with one another by branches, both macroscopic and microscopic. Likewise, it has been found that within each layer the constituting fibres anastomose freely with one another. But nevertheless, it is considered justifiable to recognise a laminar structure in the ventricles for it has repeatedly been observed by other workers and also by the candidate that a number of muscle fibres take a definite course in the ventricular wall and these groups of fibres, especially in the larger hearts, can be separated with relative ease by a method of gross dissection. Moreover, even in tiny hearts where gross dissection cannot be done successfully, cleared specimens show the same fibre arrangement as in the larger hearts. Such groups of fibres which pass in one particular direction is described in the present investigation, as a muscle layer.

PISCES

Ventricles of (a) Cod (GADUS MORRHUA).

(b) Haddock (GADUS AEGLEFINUS).

The heart of a cod or a haddock, like other teleostian fish, has a conical outlet from the ventricle which is termed the "bulbus arteriosus". There appears to be some controversy about the nomenclature of this region of the heart. Different authors have given different names to it, e.g. bulbus cordis, bulbus arteriosus and conus arteriosus. It is, therefore, considered desirable at this stage to choose an appropriate name for this part of the heart. That part of the adult heart which is developed from the embryonic anterior chamber or bulbus cordis and remains as a separate chamber in the adult, with typical cardiac muscle covering it, should be called bulbus cordis. Thus bulbus cordis is found in the Chondrichthyes or cartilaginous fish like skate, shark, etc. and in the Amphibia like frog and toad. The designation of bulbus arteriosus should be used to refer to the cranial chamber of an adult heart in which there is no cardiac muscle and which does not apparently develop from the embryonic bulbus cordis but from the backward overgrowth of the truncus arteriosus. Thus, truly speaking, it is not a part of the heart but a part of the aorta. The cranial chamber of the heart of the cod and haddock (and so also of other teleostian fish) does not contain cardiac muscle fibres, instead it is composed of plain muscle and a large amount of elastic tissue (Fig.1). This part of the teleostian heart, therefore, is designated as bulbus arteriosus.

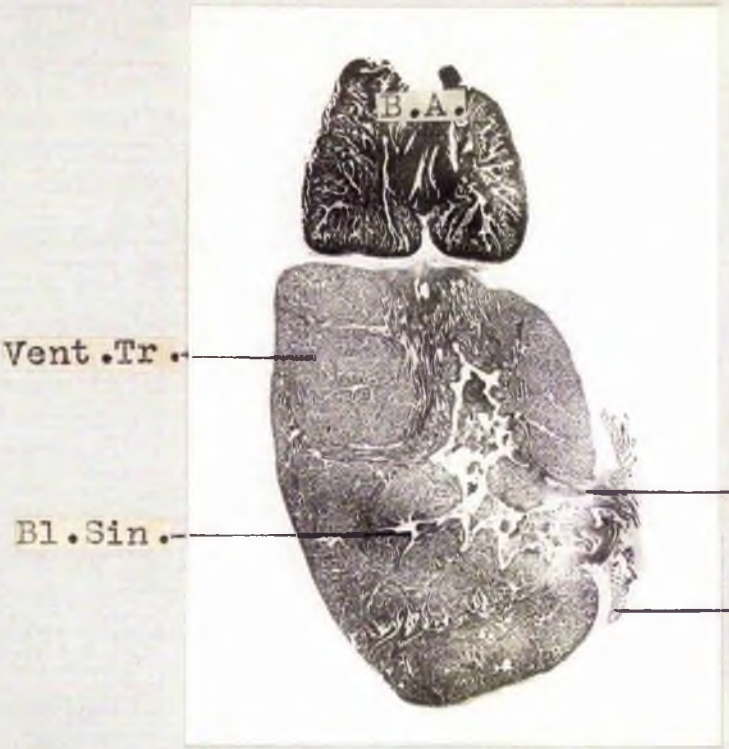

Fig.1. A sagittal section of the haddock's ventricle. Note that the bulbus arteriosus is composed predominantly of elastic tissue (intensely black) and contains no cardiac muscle fibre.

(Krajian) X 6

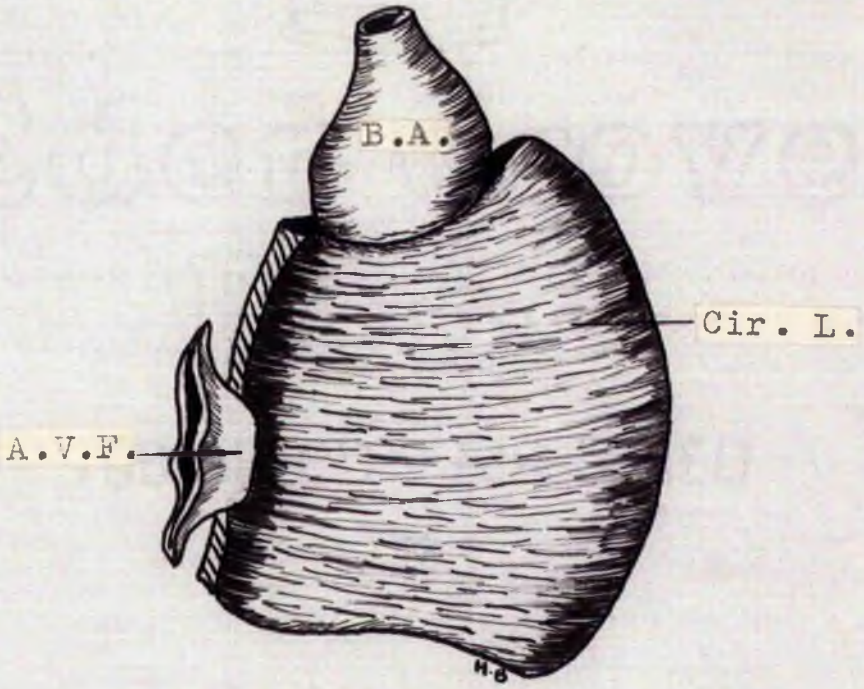

Fig.2. Drawing from a dissection of the cod ventricle showing the fibre arrangement of the superficial circular muscle layer. Viewed from the right side. X approx. 4

The ventricle of the teleostian fish consists of a superficial circular layer, deep to which there lies the trabeculated muscle which forms almost the entire thickness of the ventricular wall.

The fibres of the superficial layer (Fig.2) are in general, disposed in a circular but intricate manner. Each fibre of this layer passes superficially to the surface from the deeper muscular network and, after a variable transverse course on the surface, dips again to be continuous with the fibres of the same network from which it has originally arisen. Figs. 3 and 4 show how the fibres of the deeper trabeculae are passing to the surface to become the fibres of the superficial layer. The fibres of this layer, therefore, cannot easily be isolated on account of their connexion with the deeper muscular trabeculae. Any attempt to separate this layer from the rest of the ventricular wall leaves the surface rough and shaggy as the fibres are broken in the course of dissection. Benzoquinone stained cleared specimens showed clearly the orientation of the fibres. On account of their relatively short course on the surface, the superficial muscle layer of the cod or haddock ventricle is composed of transverse fibres which have an apparently segmented appearance. This layer is thin.

The rest of the ventricular wall is a closely woven muscular network, in which there are numerous blood spaces which communicate with the ventricular cavity. The fibres on the inner surface of the ventricle have the semblance of an inner layer which in the cod (Fig.5) is composed of apparently circular fibres but in the haddock (Fig.6) of

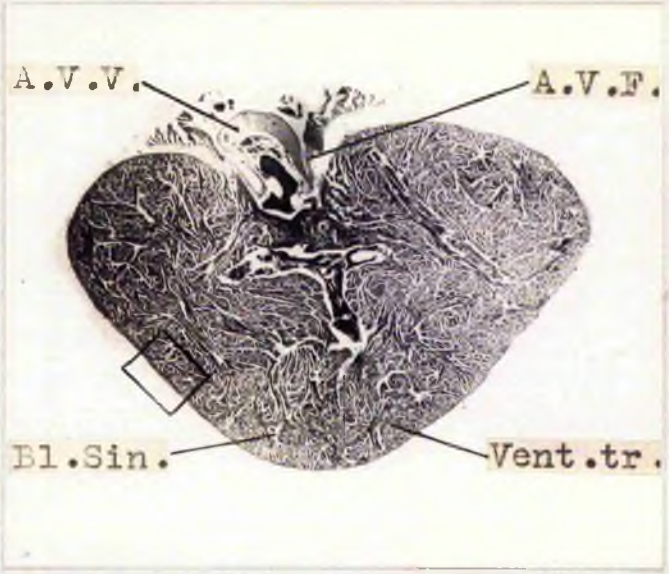

Fig.3. A transverse section of the haddock's ventricle. Shows that the fibres of the inner trabeculae pass to the surface to form the superficial circular muscle layer.

(E.H.) X 10

Fig.4. Higher magnification of the enclosed portion of Fig.3. Shows that the superficial circular muscle layer lies immediately beneath the epicardium.

(E.H.) X 110

longitudinal fibres. Around the A.V. opening the ventricular wall is slightly invaginated and in this region the superficial layer becomes continuous with the inner muscular network as well as with the ventral end of the structure which has been designated the "A.V. funnel" (Figs. 3 and 5). The A.V. funnel is a cone-shaped prolongation of the atrial musculature through A.V. opening into the ventricle. The funnel passes dorso-ventrally on its way from atrium to ventricle and at its ventral end its fibres are continuous with those of the ventricular muscular network as well as with those of the invaginated ventricular wall. These connecting fibres encircle the A.V. orifice and establish a muscular connexion between the atrium and the ventricle, whose two musculatures are, therefore, to be regarded as continuous with each other. The fibres of the muscular network do not appear to be arranged in any definite pattern as they run in all directions with the exception of those which pass circularly around the A.V. opening.

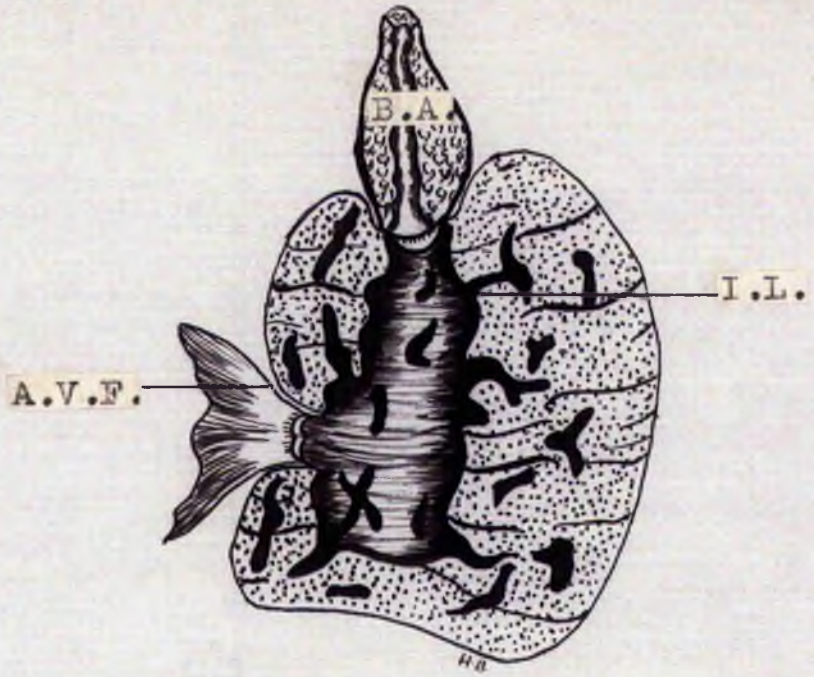

Fig.5. Drawing of the mid-sagittal section of the cod heart showing the fibre disposition of the inner layer of the ventricle and the continuity of the A - V funnel with the invaginated ventricular wall.

X approx. 3

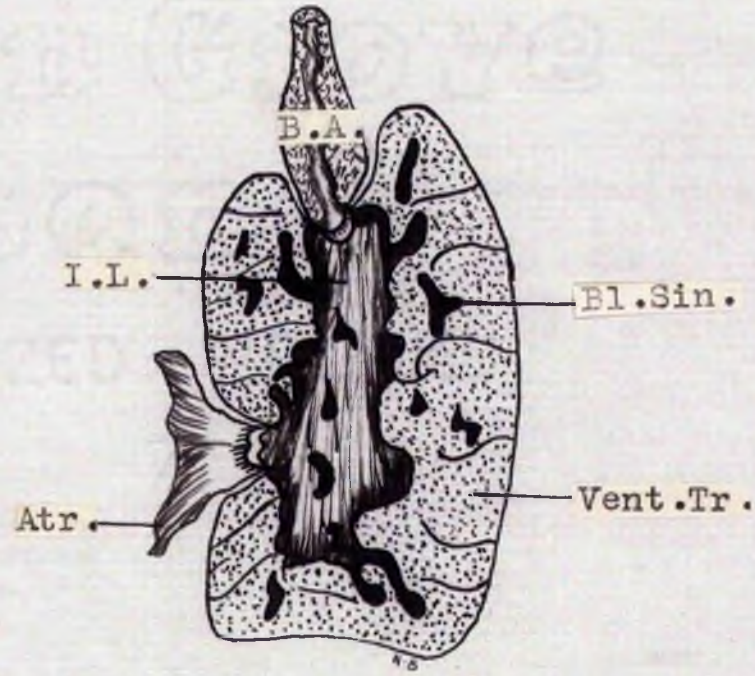

Fig.6. Drawing of the mid-sagittal section of the haddock's heart showing the fibre disposition of the inner layer of the ventricle and the continuity of the A - V funnel with the invaginated ventricular wall.

X approx. 4

AMPHIBIA

Ventricle of the common British frog. (RANA TEMPORARIA)

The ventricular musculature of the frog's heart consists of a thin superficial circular layer and a dense inner muscular trabecular network. The superficial circular layer (Fig.7) lies immediately deep to the epicardium. It is very similar in appearance and in fibre arrangement to the superficial circular layer of the fish ventricle. This layer is extremely thin and can not be separated cleanly by dissection from the rest of the ventricular musculature. But its fibre arrangement could be beautifully seen under the binocular microscope especially in cleared specimens after staining with Benzoquinone. This layer is formed by the fibres which come to the surface from the deeper trabecular network and pass transversely for some distance on the outer surface of the ventricle before they pass back to the depth where they again become continuous with the fibres of the inner trabeculae. The fibres of this layer as seen on the superficial surface of the heart are, therefore, apparently segmented. The apparent segmentation of the fibres of this layer was beautifully seen in cleared specimens as well as in microscopic sections.

The main thickness of the ventricle of the frog is constituted by the inner trabeculae which, similar to the fish, are composed of a meshwork of muscle strands (Fig.8). Near the A.V. opening they join the caudal margin of the A.V. funnel which is a cone-shaped extension of the atrial muscle into the ventricle. Around the A.V. junction

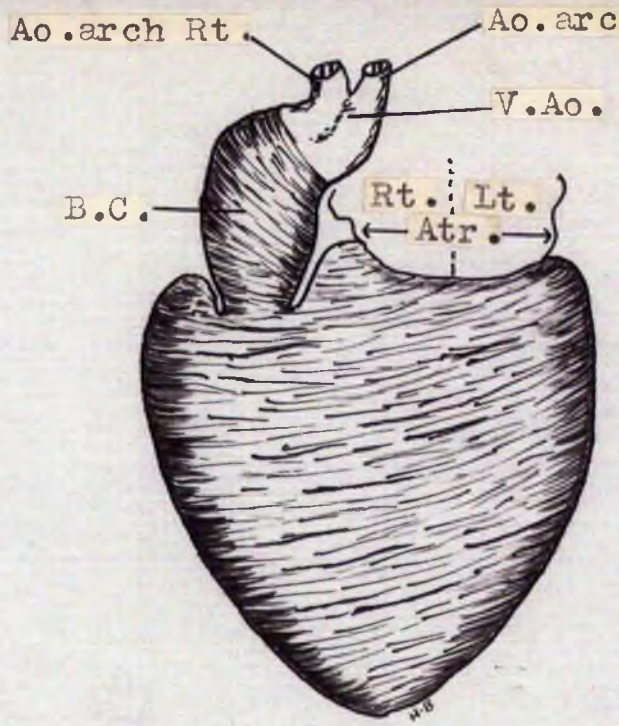

Fig.7. Drawing from a cleared specimen of the frog heart, showing the fibre arrangement of the superficial muscle layer of the ventricle and bulbus cordis. Ventral view.

X approx. 8

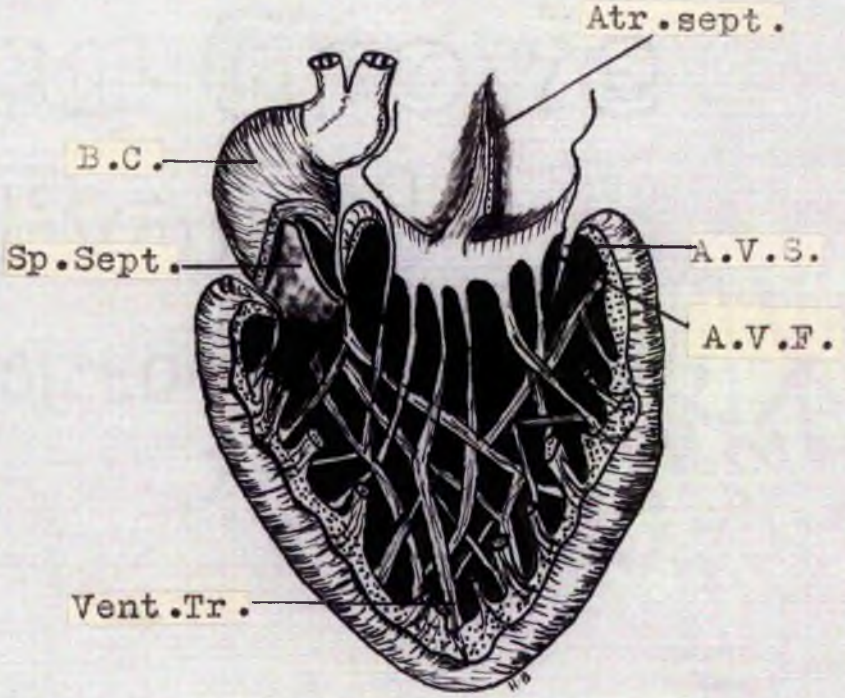

Fig.8. Drawing of the interior of the frog's ventricle showing the trabecular network. Continuity of the A - V funnel with the trabeculae is clearly seen.

X approx. 8

the base of the ventricle is invaginated and there the invaginated portion of the ventricular wall becomes continuous with both the caudal margin of the A.V. funnel and also with the ventricular trabecular network. Although there is this continuity between the musculatures of the atria and the ventricle, there is, nevertheless, a deep atrio-ventricular sulcus on the superficial aspect of the heart and this is shown in Fig.8.

Towards the apex of the ventricle the inner trabeculae divide and subdivide and the finer divisions interdigitate with one another in such a manner that a dense muscular meshwork is formed (Fig.8). All around the ventricular wall fibres of the trabecular network come out to the surface and form the superficial circular layer already described. Although the trabeculae form a dense irregular meshwork, and especially so close to the apex, it was found that as the basal region of the ventricle was approached the fasciculi became arranged somewhat longitudinally and as already described, were continuous with the A.V. funnel and the invaginated ventricular base. It appears, therefore, that the muscle fibres of the trabeculae in the frog are more organised than those in the fish, in which they are rather haphazardly arranged.

The bulbus cordis of the frog heart is a twisted somewhat cylindrical chamber (Fig.9) which arises from the right upper part of the ventricle. From its origin it bends slightly to the left and then passes upwards. In the upper end, it becomes continuous dorsally with the short ventral aorta. The ventral aorta divides into a right

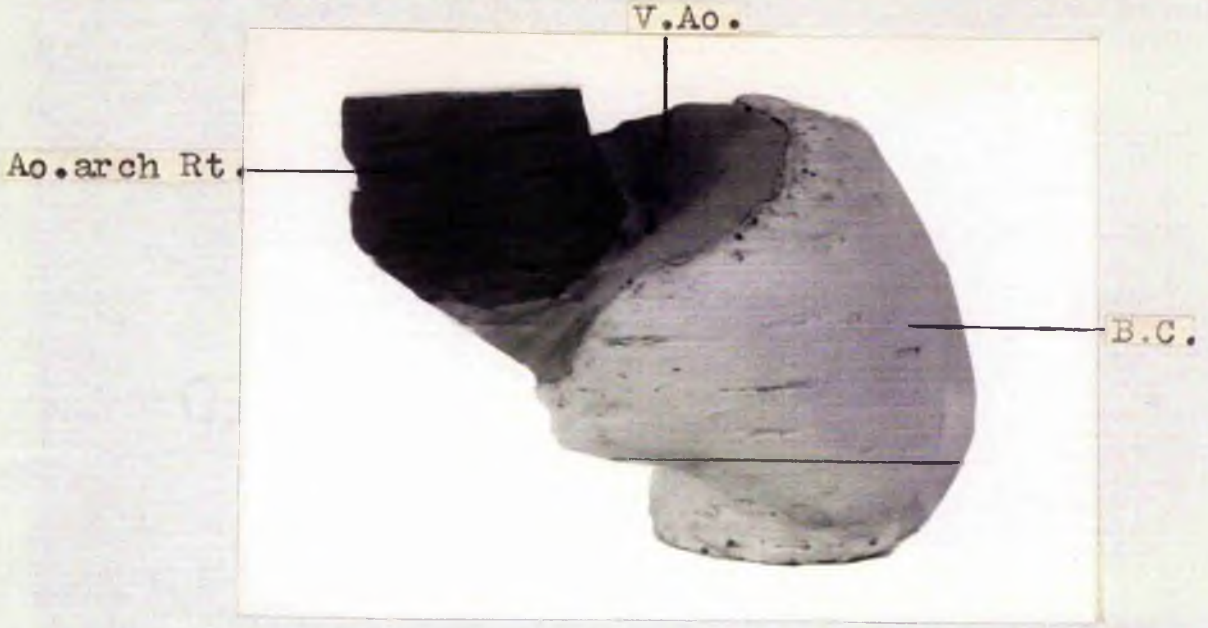

Fig.9. Reconstruction of the bulbus cordis of the frog's heart, viewed from the right side. The bulbus has the typical form and the short ventral aorta is seen emerging from the cranial end of its dorsal surface. The left aortic arch is hidden by the right arch.

X 17

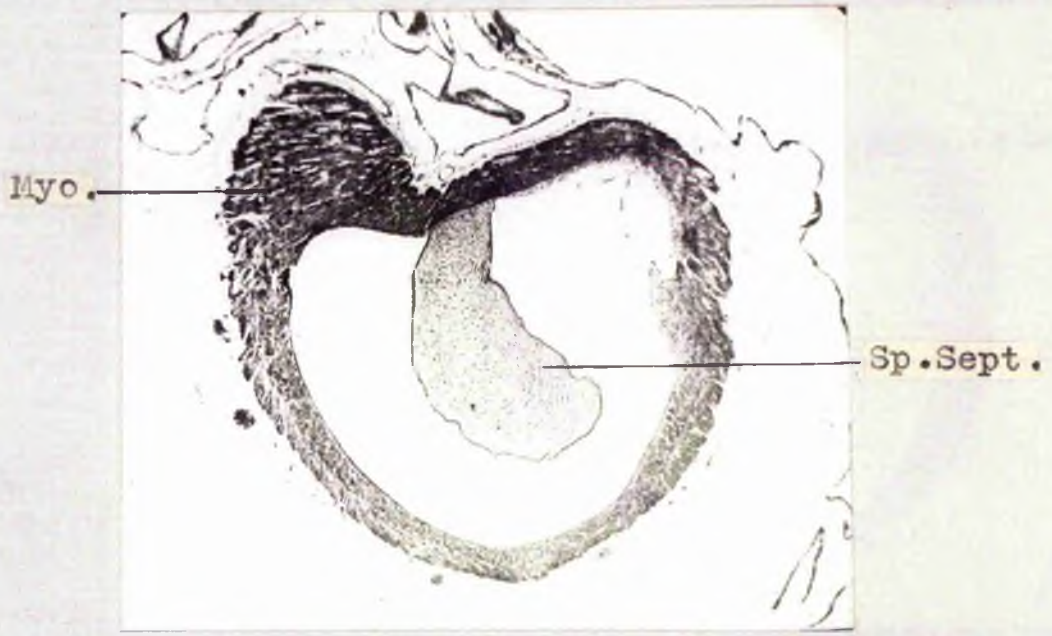

Fig.10. Microphotograph of a transverse section of the bulbus at a level shown in Fig.9. Note the typical cardiac muscle covering of the bulbus cordis.

(E.H.) X 35

and a left aortic arch, each of which in turn gives rise to the three main arteries for each half of the body. The ventral wall of the bulbus cordis is longer than its dorsal wall, as a result of which the bulbo-aortic junction looks backwards and slightly downwards.

The bulbus cordis of the frog is contractile and is covered with a compact layer of cardiac muscle, the fibres of which are arranged in a circular manner (Fig.10).

Around the bulbo-ventricular junction the ventricular base is slightly invaginated by the bulbus cordis so that in this site it is the distal chamber which invaginates the proximal and not vice versa, as at the A.V. junction. At the caudal margin of the invagination, the musculature of the two chambers has been verified microscopically to be continuous. The continuity is established by longitudinal strands of muscle fibres of the ventricular trabeculae which, passing cranially to the bulbus, become continuous with its circular muscle fibres.

REPTILIA

Ventricle of the green lizard. (LACERTA VIRIDIS)

Similar to the two monoventricular hearts already described, ventricle(s) of the lizard consists of a thin superficial layer and dense inner muscular trabeculae. The superficial layer lies immediately deep to the epicardium and its fibres are arranged almost circularly although there is a slight but definite spiral twist towards the left. This spiral arrangement is well marked in the cranial two thirds of the ventral surface. On this surface the fibres of the superficial layer arise from the ventral margin of the A.V. opening and the roots of the great vessels (Fig.11) and winding round the left margin of the ventricle reach the caudal part of its dorsal surface. On the dorsal surface the fibres of the superficial layer arise from the left and dorsal margin of the A.V. opening and spreading over the surface with a slight anti-clockwise twist, wind round the right margin to reach the caudal part of the ventral surface. The superficial fibres on the cranial parts of both dorsal and ventral surfaces arise from the basal structures of their own surfaces whereas the superficial fibres on the caudal parts of both ventral and dorsal surfaces arise from the basal structures of the opposite surfaces. The superficial fibres terminate at varying distances from their origin by passing into the depth and joining the inner trabeculae. Fibres of this layer, on the dorsal surface of the ventricle, are more transversely disposed than those on the ventral surface and some fibres near the apex

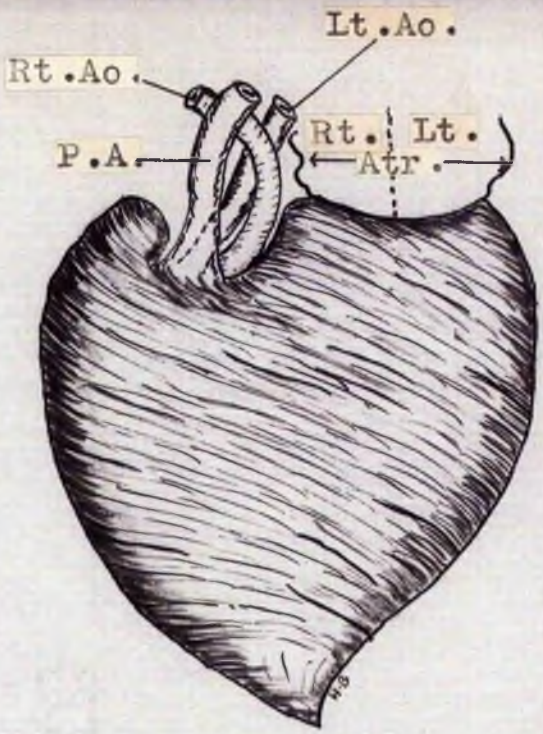

Fig.11. Ventral view of the lizard's ventricle, drawn from a stained cleared specimen. The superficial muscle fibres are disposed with slight spiral inclination to the left.

X approx. 8

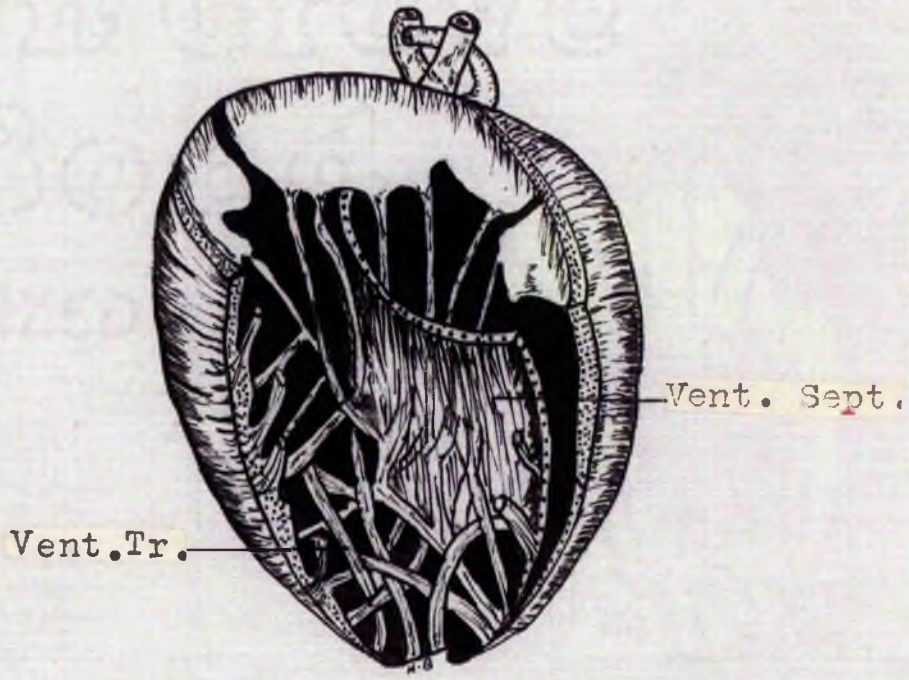

Fig.12. Interior of the lizard's ventricle, opened from the dorsal surface, showing the inner trabeculae and the incomplete ventricular septum.

X approx. 8

appeared to be arranged almost vertically on the ventral surface.

Beneath the superficial layer there is the dense muscular trabeculae which constitute the major part of the ventricular wall. In the basal portion of the ventricle(s) they are arranged along its long axis (Fig.12). These trabeculae in the lizard are more numerous and better developed than those in the frog. They are attached cranially to the invaginated ventricular base around the A.V. opening and also to the margin of the A.V. funnel and A.V. valves. Caudally towards the apex they divide, sub-divide and interdigitate to form a close network.

The ventricle(s)of the lizard is incompletely divided into a right and a left half by a septum, the cranial margin of which is concave and free (Fig.12) and leaves a passage over it, through which the two halves of the ventricle communicate. The ventricular septum appears to be formed by the fusion of the trabeculae and most of its fibres are arranged longitudinally. There is no bulbus cordis in the reptilian heart because the embryonic bulbus is divided and forms the proximal parts of the three main arteries which arise from the ventricle.

Ventricle of the grass snake. (NATRIX NATRIX)

The shape of the heart of the grass snake is elongated which might be considered as being in accordance with its long slender body and it is orientated so that its long axis is approximately in the long axis of the animal.

The ventricular wall of the grass snake heart has two distinct layers

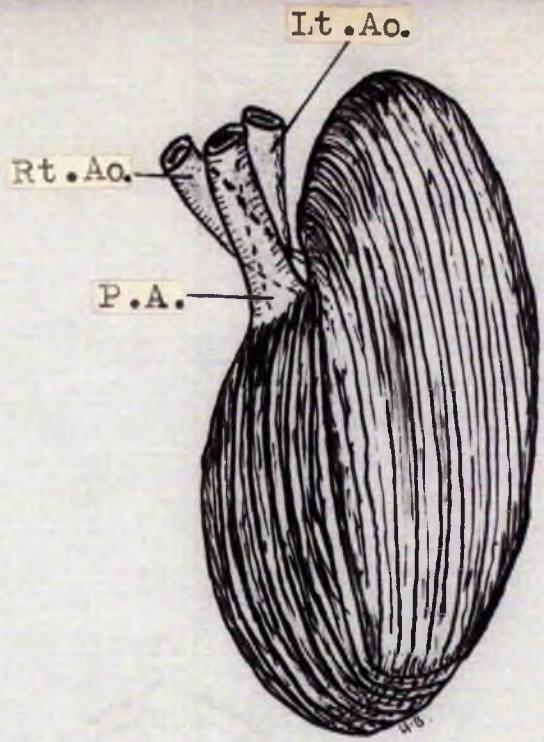

Fig.13. Ventral view of the superficial longitudinal muscle layer of the snake ventricle. Note that the fibres of this layer are arranged in two strata close to the apex; they cross one another approximately at right angles.

X approx. 6

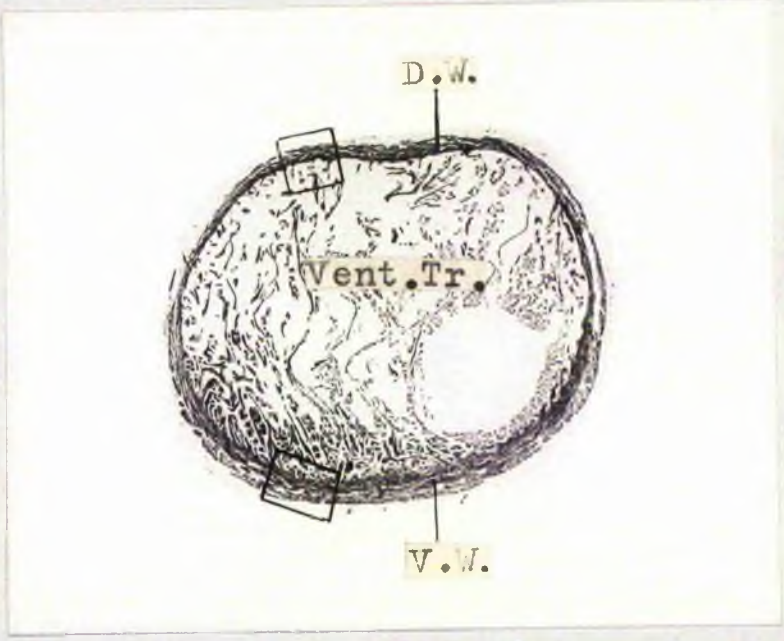

Fig.14. Microphotograph of a transverse section of the snake ventricle showing the different thicknesses of its ventral and dorsal wall.

(E.H.) X 15

which, on dissection, are easily separated from each other. The superficial layer is formed by loops of longitudinal fibres which on the ventral surface have cranial attachments to the roots of the pulmonary artery and the left aorta and to the ventral and right margin of the A.V. opening (Fig.13). From this extensive cranial attachment, fibres pass caudally and slightly to the left in the ventral wall of the ventricle(s) and turn round the apex of the heart to reach the dorsal surface where they are attached to the root of the right aorta and the dorsal and left margin of the A.V. opening. Fibres on the left side, on their way back to the base, form loops around the cranial end of the left half of the ventricle(s). Although the superficial longitudinal fibres of the ventricle(s) of the snake run parallel to one another and lie in the same plane over the greater extent of the ventricle(s), there is a region adjacent to the apex, where these fibres form superficial and deep strata; these strata cross each other in the region of the apex approximately at right angles (Fig.13). Fibres coming from the ventral and right margin of the A.V. opening form the deep stratum and pass deep to the fibres coming from the arterial roots, which thus forms the superficial stratum. The superficial longitudinal layer varies in thickness in different parts of the ventricular wall, being approximately three times thicker on the ventral wall than on the dorsal. The fibres are also more compactly arranged in the ventral wall than in the dorsal where the layer is not only thin but also loose in texture; the superficial fibres of the dorsal wall are separated from one another

Fig.15. Higher magnification of the enclosed area of the dorsal wall as shown in Fig.14. Note that the superficial muscle layer is incomplete in the left extremity of the photograph.

(E.H.) X 50

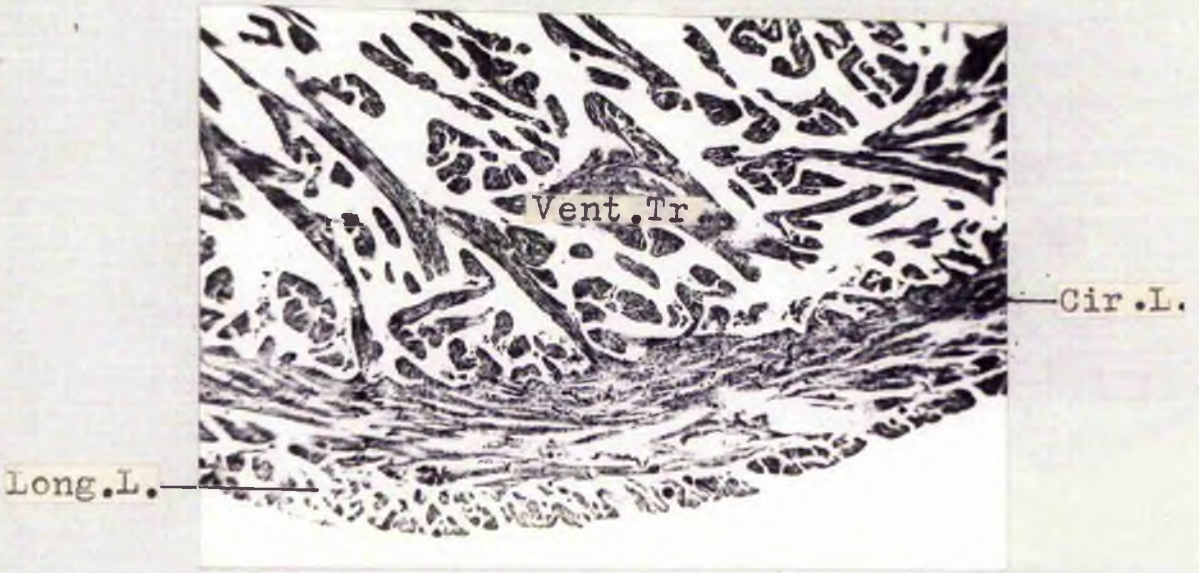

Fig.16. Higher magnification of the enclosed area of the ventral wall as shown in Fig.14.

(E.H.) X 50

and through the gaps fibres of the deeper layer come in contact with the epicardium. Transverse microscopic sections show clearly the different thicknesses of the superficial layer in the two walls and also the incompleteness of the layer in the dorsal wall (Figs. 14, 15 and 16).

Immediately below the superficial longitudinal layer lies the second layer of the ventricular wall and its fibres are arranged circularly with a slight but definite spiral disposition (Figs.17 and 18). Fibres of this second layer, like those of the circular layer of the other ichthyopsidan ventricle, are not continuous all around the ventricular wall but are formed of segments of fibres which come to the surface from the deeper trabeculae and traverse a variable distance in a circular manner and return to the depth where they are again continuous with the trabecular network. Because of this disposition of the fibres this layer cannot be dissected with ease like the superficial longitudinal layer. Segmented appearance of the fibres of this layer was clearly visible in the stained cleared specimens. Transverse microscopic sections (Figs. 14 - 16) show dipping in of the fibres and their continuation with the inner trabecular network.

On the ventral surface, fibres of the circular layer flow from the right to the left with a slight spiral inclination caudally (Fig.17). Fibres on the dorsal surface take a similar course but in the opposite direction (Fig.18). Around the A.V. orifice the ventricular base is slightly invaginated. The caudal margin of the invagination is continuous with the caudal margin of the A.V. funnel and also with

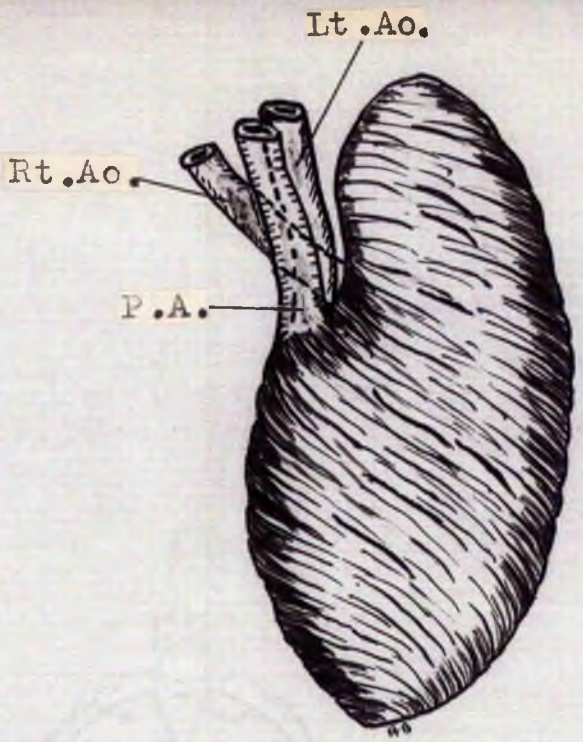

Fig.17. Drawing from a dissection of the grass snake heart showing the fibre arrangement of the second layer on the ventral surface of the ventricle. The spiral disposition of its fibres is clearly seen.

X approx. 6

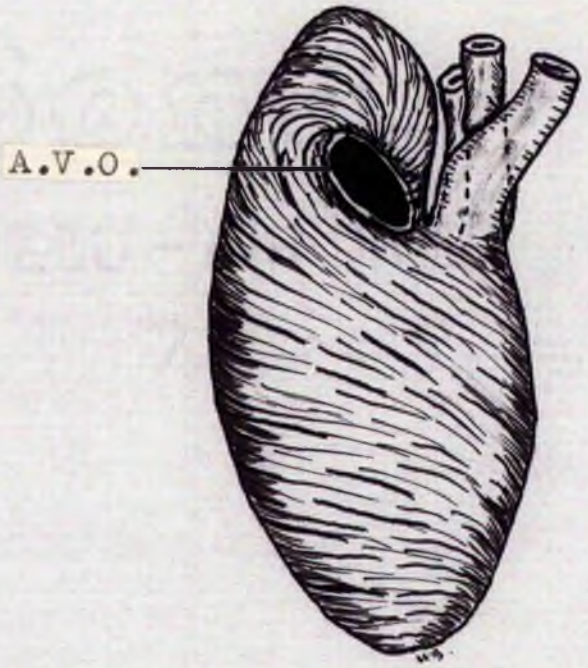

Fig.18. Arrangement of the muscle fibres of the second layer on the dorsal surface of the ventricle of the grass snake.

X approx. 6

the inner trabecular network. The circular layer, similar to the longitudinal layer, is also thicker in the ventral wall than in the dorsal (Figs.15 and 16). The spiral character of the fibres of this second layer of the snake ventricle(s) is more marked than that of the fibres of the circular layer of the lacertilan ventricle(s).

The interior of the ventricle(s) is covered with muscular trabeculae, the majority of which are arranged along the long axis of the ventricle(s). This regular arrangement of the trabeculae is better seen in the basal half of the ventricle(s) where, adjacent to the A.V. opening, they become continuous with the caudal margin of the A.V. funnel. Towards the apex of the ventricle(s) trabeculae form a dense muscular meshwork. It is the fibres of these trabeculae which penetrate to the surface and help to form the circular layer already described.

The ophidian ventricle(s), similar to that of the lizard, is partially divided by an incomplete septum, and again the cranial margin is free and concave and over which the two halves of the ventricle freely communicate with each other. The muscle fibres of the septum are arranged longitudinally and are continuous in the apical region with the trabecular network. The right half of the ventricle is further sub-divided into a larger ventral and right part and a smaller dorsal and left part by a second small incomplete septum which apparently is a prolongation into the cavity, of the septum which separates the root of the pulmonary artery from that of the left aorta.

AVES

Ventricles of the domestic fowl. (GALLUS DOMESTICUS)

As the muscle layers of the avian ventricles are arranged in a manner very similar to that found in the mammalian heart, the same terminology as used by Mall (1911) in his description of the mammalian ventricular muscle layers, is therefore used in the present investigation in the bird. The name of one layer, the longitudinal muscle of the right ventricle is, however, changed to longitudinal layer of the ventricular septum as the latter term gives a clearer idea of its situation in the ventricular wall.

For the convenience of description, the muscle layers of the avian ventricles may be classified on the topographical basis of their origin into the following groups:-

1. Bulbospiral group
   - (a) Superficial bulbospiral layer.
   - (b) Deep bulbospiral layer.
   - (c) Longitudinal layer of the ventricular septum.
   - (d) Circular layer of the left ventricle.
2. Sinospiral group
   - (e) Superficial sinospiral layer.
   - (f) Deep sinospiral layer.

All the ventricular muscle layers are attached to the fibro-cartilaginous skeleton of the heart, and a complete view of this may be obtained, when the epicardium fat, blood vessels and nerves are

removed and the atria are detached from the ventricles (Fig.19). The fibrocartilaginous skeleton consists of the following:-

(i) Trigonum fibrosum dextrum:- The right trigone is a mass of fibrous tissue situated between the two A.V. openings and behind the root of the aorta (Fig.19). It is somewhat triangular in shape having its base towards the aortic root with which it is continuous. Its apex is directed dorsally and is continuous with the left annulus fibrosus. A part of the right margin of the trigone is continued into the right annulus fibrosus.

(ii) Trigonum fibrosum sinistrum:- The left trigone is a similar but smaller triangular fibrous mass, situated at the left side of the aorta. Its base is at the aortic root and the apex is continuous with the left annulus fibrosus.

(iii) Annuli fibrosi:- These are two fibrous rings surrounding the A.V. openings and these have different consistencies in different parts. Sometimes they are fibrocartilaginous close to the trigona but in the rest of the circumference they are fibrous and thin.

(iv) Conus tendon:- This is a fibrous band (Fig.19) which connects the roots of the pulmonary artery and the aorta and extends forwards and to the left from the aorta to the pulmonary artery.

(v) Fibrous rings of the arterial roots:- These are two fibrous rings which encircle the aorta and the pulmonary artery at their origins from the ventricles.

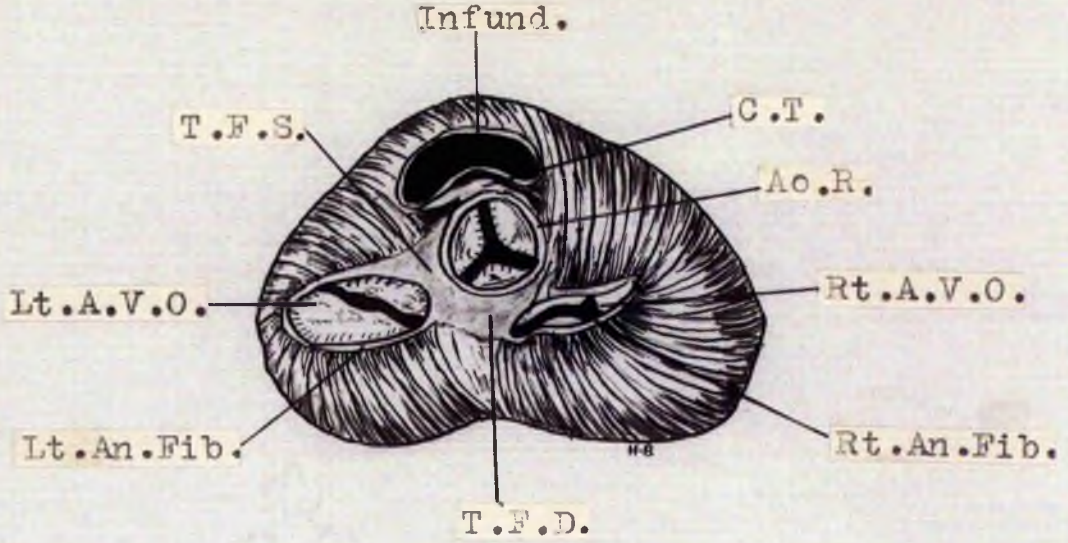

Fig.19. A view of the cardiac skeleton of the fowl heart as seen after removal of the atria and the great vessels. It is to be noted that in the bird, as in mammals, the aortic valve is at a lower level than that of the pulmonary artery and for this reason the infundibulum was cut across in the specimen from which the drawing was made.

X approx. 2½

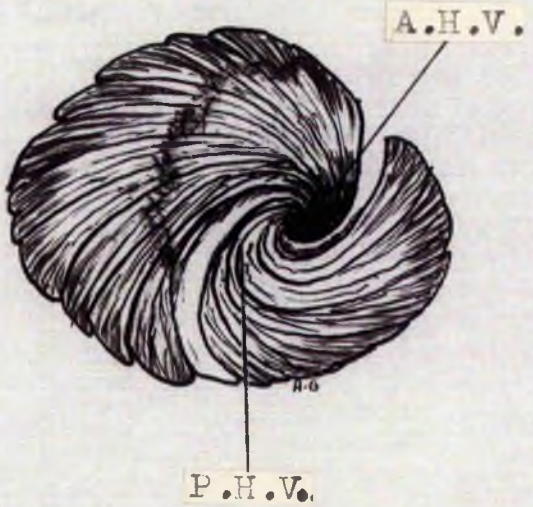

Fig.20. A view of the arrangement of the muscle fibres at the apex of the fowl heart showing the anterior and the posterior horns of the vortex.

X approx. 10

When the epicardium, fat, blood vessels etc. are removed from the ventricles - both superficial muscle layers come under view.

Superficial bulbospiral layer (Figs.21 & 22):- Fibres of the superficial bulbospiral layer are attached to the conus tendon, pulmonary root, trigonum sinistrum, ventral and left parts of the left annulus fibrosus. Minor variations of this attachment were observed in different specimens. In one heart it did not extend to the conus tendon while in another the attachment extended to the entire dorsal margin of the left annulus. From this extensive origin the fibres pass caudally and to the left in an anti-clockwise fashion and converge towards the apex to form the posterior horn of the vortex, in which the fibres are aggregated into a thicker but narrower band. At the apex of the heart, fibres turn upon themselves and enter the interior of the left ventricle whence they ascend cranially and spread out in the inner surface of its posterior wall and are ultimately inserted into the posterior and medial margins of the left annulus. Inside the left ventricle they also help to form the small posterior papillary muscle. The continuation of the superficial bulbospiral layer on the inner surface of the posterior wall of the left ventricle may clearly be seen when that ventricle is opened out. Thus it appears that the fibres of this layer descend on the outer surface of one wall of the ventricle and ascend on the inner surface of the opposite wall and furthermore they describe an open figure of eight (8) in their course from the origin to the insertion. This layer is thin and the fibres are almost longitudinally arranged and can be separated easily

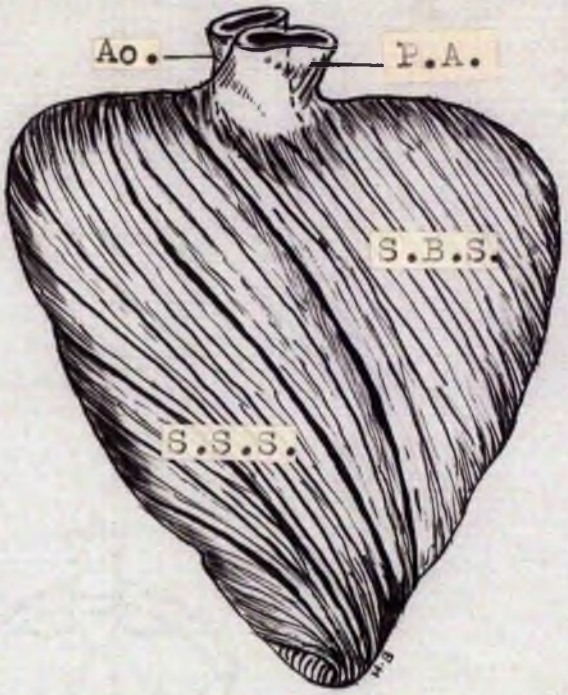

Fig.21. A view of the sternocostal (ventral) surface of the fowl ventricles showing the disposition of the muscle fibres of the superficial layers.

X approx. 2½

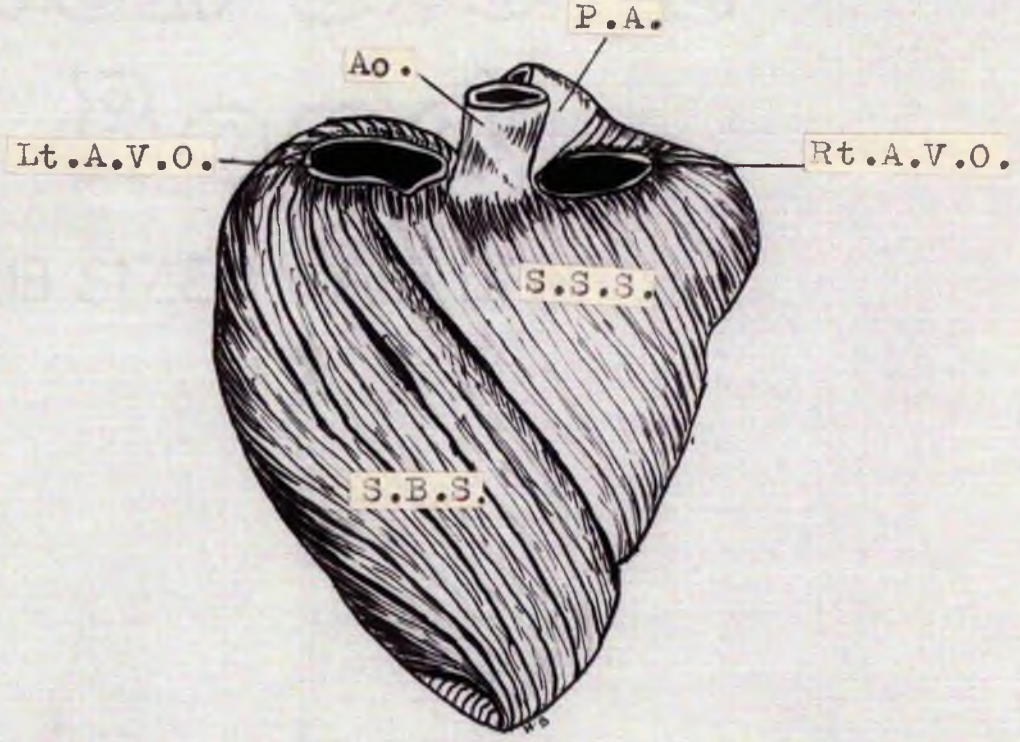

Fig.22. Diaphragmatic (dorsal) surface of the fowl ventricles showing the fibre arrangement of the superficial layers.

X approx. 2½

from the deeper layers by ordinary dissection.

Superficial sinospiral layer (Figs.21 & 22):- This layer takes its origin from all round the right annulus fibrosus, trigonum dextrum and a small part of the posterior margin of the left annulus. From this attachment fibres pass caudally and to the right and after winding round the right border of the heart, they gain the ventral (sternocostal) surface where the fibres are aggregated adjacent to the apex and form the anterior horn of the vortex and turn upon themselves to gain the inner surface of the left ventricular wall. Inside the ventricle fibres ascend subendocardially in its anterior and the left walls and are finally inserted into the left trigone and the adjacent part of the left annulus. They also help to form the anterior papillary muscle of that ventricle. This layer, similar to the superficial bulbospiral layer, is thin and its fibres likewise describe an open figure of eight (8).

Some of the fibres of both superficial bulbospiral layer and superficial sinospiral layer, while crossing over the ventral and dorsal interventricular grooves, penetrate through these grooves and gain the ventricular septum. These perforating fibres are more numerous in the ventral than in the dorsal groove. An experience of this was the greater difficulty encountered in separating the superficial layers from the deeper layers in the ventral groove. As the fibres of both superficial layers lie in the same plane on the ventricular wall and are arranged almost longitudinally, it is difficult to determine the lines of demarcation between the two layers and especially

in the basal region; these lines can, however, be easily determined if the two horns of the vortex are followed from the apex to the base. Fig.20 shows the orientation of the fibres in the two horns of the vortex, which belongs only to the left ventricle. There is no vortex in the right ventricle.

Deep bulbospiral layer (Fig.23):- Fibres of this layer arise from an area of the ventricular base corresponding to the origin of the superficial bulbospiral layer but situated in a deeper plane. Its fibres are coarser and more fascicular in appearance, more transverse and markedly spiral in disposition than those of the superficial layers. From this attachment the fibres pass caudally and to the left and winding round the left margin of the heart, reach the caudal half of the dorsal (diaphragmatic) surface where some fibres penetrate deep into the ventricular wall through the dorsal interventricular groove. The majority of the fibres, however, reach the ventral surface. A few fibres on this surface also pierce the ventral interventricular groove to ascend in the opposite direction in the ventral wall of the right ventricle and the ventricular septum to be attached to the anterior and right margin of the right annulus fibrosus. The remainder of the fibres cross the ventral interventricular groove to reach the left ventricle, where they blend with the fibres of the longitudinal layer of the ventricular septum, which will be described later, and are inserted on the endocardial surface of the left ventricle along with them. The deep bulbospiral layer is, in some places, firmly adherent to the deeper circular layer of the left

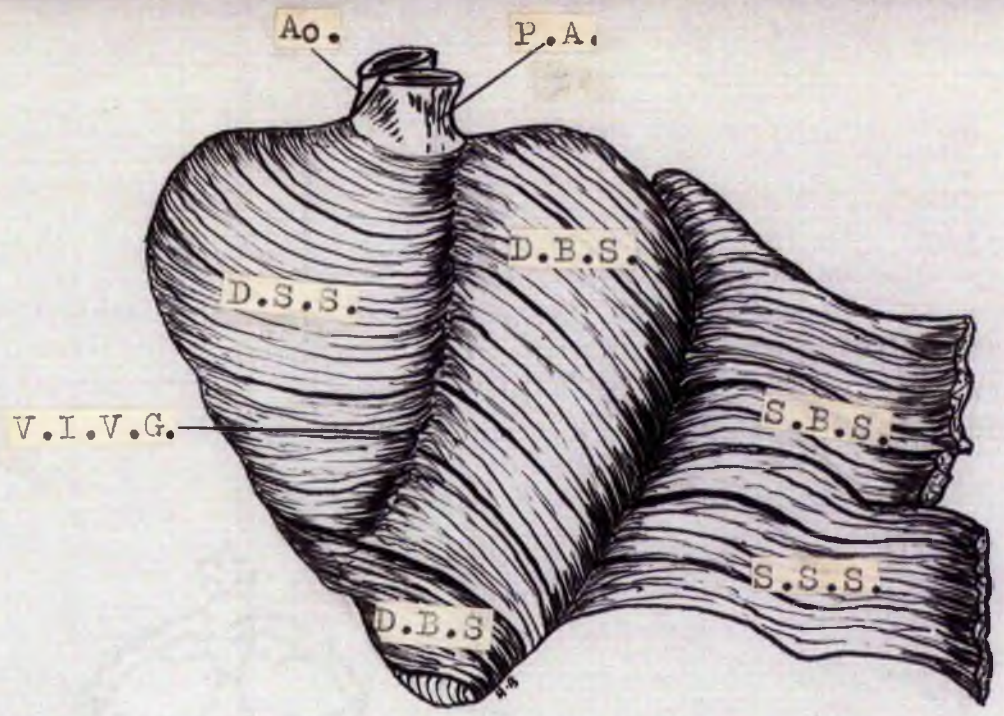

Fig.23. A view of the sternocostal surface of the fowl ventricles, after reflection of the superficial muscle layers. It is to be noted that most of the fibres of the deep sinospiral layer penetrate through the ventral interventricular groove.

X approx. 2½

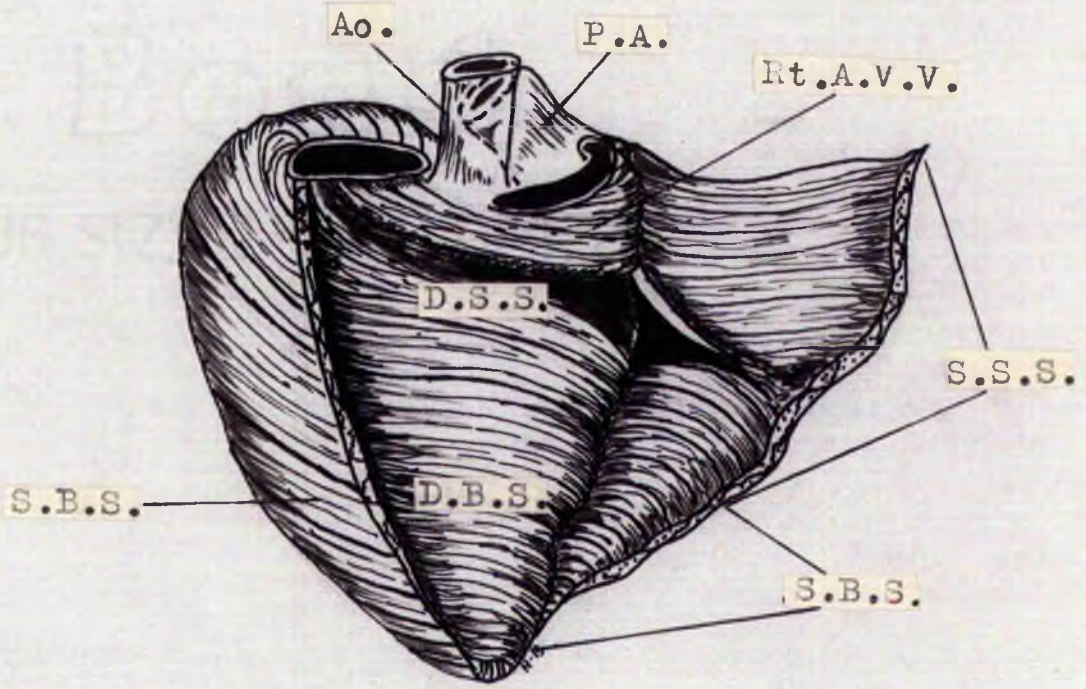

Fig.24. Dorsal (diaphragmatic) surface of the fowl heart after reflecting a part of the superficial layers. Note that the fibres of the deep sinospiral layer form the right A.V. valve.

X approx. 2½

ventricle making separation of the two layers difficult. Those fibres of this layer, which take origin from the right half of the conus tendon form loops over the infundibulum and return back to the left half of the conus tendon and the left trigonum fibrosum (Fig.23).

Deep sinospiral layer (Figs. 23, 24 & 25):- This layer which is similar to the previous one, is attached to an area corresponding to the origin of the superficial sinospiral layer but on a deeper plane. Fibres from this origin pass caudally and to the right and after winding round the right border of the heart reach the ventral (sternocostal) surface of the right ventricle. In the ventral interventricular groove, most of the fibres penetrate deep into the ventricular wall and ascend in the ventricular septum and in the deeper surface of the ventral wall of the right ventricle, to be finally attached to the right annulus fibrosus and the pulmonary root. Only a few superficial fibres of this layer cross the ventral inter: :ventricular groove and passing to the left ventricle there blend with the fibres of the longitudinal layer of the ventricular septum and are inserted along with them. Throughout the course of this layer, fibres constantly pass into the deeper surface of the ventricular wall whence they ascend towards the right annulus. Some deeper fibres of this layer, originating from the trigonum dextrum, encircle the right A.V. opening and are attached ventrally to the conus tendon. This group of fibres of the deep sinospiral layer constitutes the muscular right atrioventricular valve, a structure peculiar to the avian heart (Fig.24). This band of fibres becomes very evident when an attempt

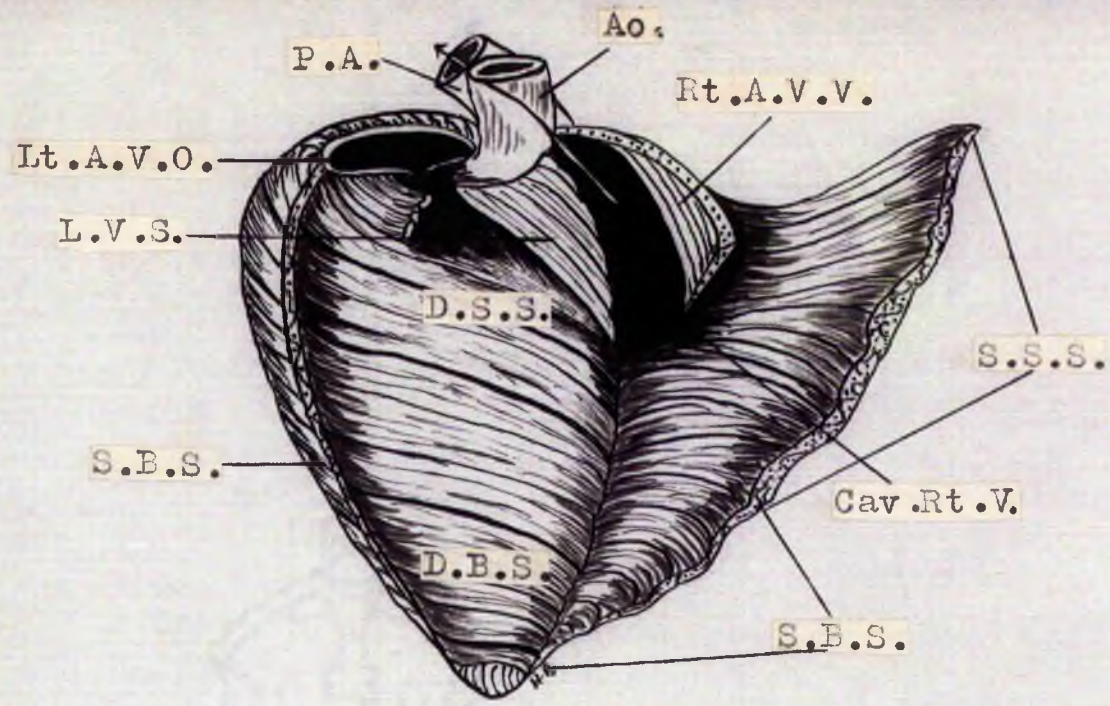

Fig.25. The same view as in Fig.24, after the right A.V. valve has been cut. This permits further unrolling of the deep sinospiral layer and the complete cavity of the right ventricle is now exposed and the origin of the L.V.S. is seen in the upper part of the septum.

X approx. 2½

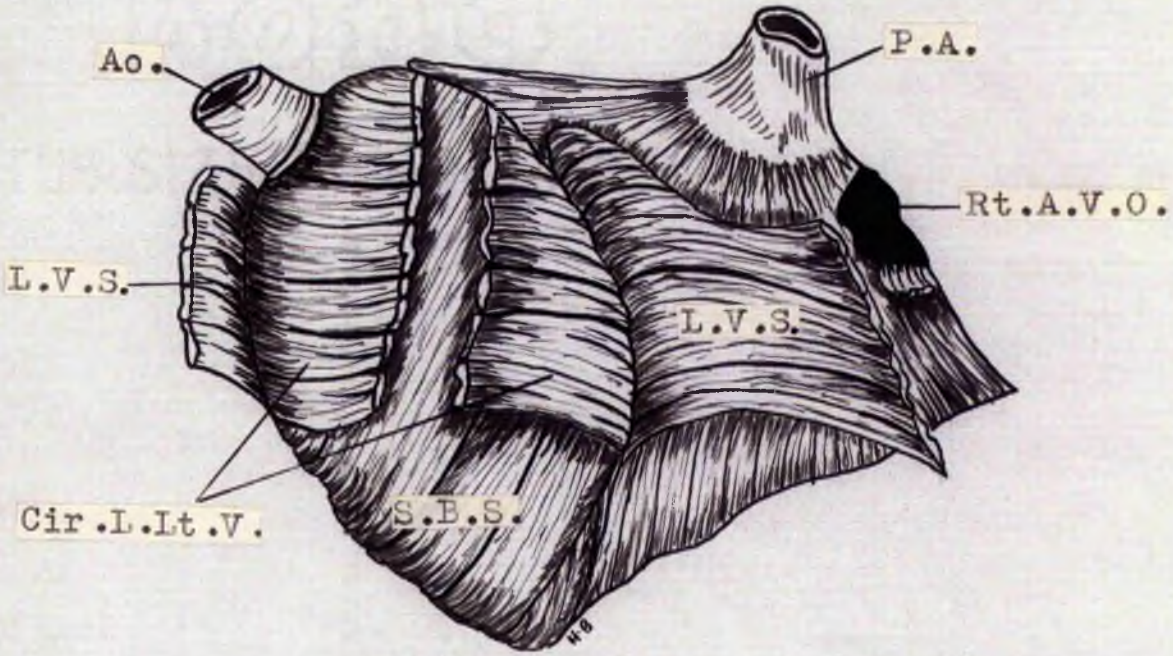

Fig.26. A view of the circular layer of the left ventricle of the fowl heart. Ventricles have been cut and unrolled from the dorsal aspect.

X approx. 2½

is made to separate the deep sinospiral layer from the deeper layers along an incision on the dorsal surface of the left ventricle close to its left border (Fig.24). Further separation of the layer is, however, prevented by the band of fibres going to the right A.V. valve. When this band is cut, further unrolling is then possible and the cavity of the right ventricle is fully exposed (Fig.25).

Longitudinal layer of the ventricular septum (L.R.V. of Mall, 1911) (Figs.25 and 26)

This layer along with some of the ascending fibres of the deep sinospiral layer form the septal wall of the right ventricle. Its fibres have origin from the trigonum dextrum and the front and right side of the aortic root from which they pass spirally along the ventricular septum towards the apex of the heart. They gain the ventral (sternocostal) surface of the left ventricle through the ventral interventricular groove where they merge with the fibres of the deep bulbospiral layer and with them enter into the substance of the wall of the left ventricle through the posterior horn of the vortex; in the terminal part of their course they lie deeply in the substance of the wall. As they lie in the deep substance of the wall, they ascend in the opposite direction in the dorsal and left walls of the left ventricle and are finally inserted into the left annulus and the left trigone.

Circular layer of the left ventricle (Fig.26):- This is a thick layer and the fibres are circularly disposed. The layer is confined to the left ventricle and occupies approximately half of its length from base to apex. It is situated deep to the origins of the other layers and becomes visible when the right ventricle is unrolled away

from the left ventricle by an incision along the dorsal interventricular groove and the longitudinal layer of the ventricular septum is also peeled away along with the right ventricle (Fig.26). The lower margin of the circular layer is well defined, deep to which the fibres of all superficial layers may be seen gaining the deeper surface of the left ventricular wall. Fibres of this circular layer are attached to the whole circumference of the left annulus and pass almost horizontally in an anti-clockwise manner. In their course in the ventricular wall, the superficial fibres of this layer, turn upon themselves to become deep fibres of the layer and are inserted to the same left annulus fibrosus. This layer is the thickest of all layers and its fibres are coarser and fascicular. When this layer is severed, the whole left ventricle can be opened out like a flat sheet of muscle.

MAMMALS

Ventricles of the (a) Ox, (BOS TAURUS).

(b) Sheep, (OVIS ARIES).

(c) Pig, (SUS DOMESTICUS).

The muscular architecture of the mammalian ventricles, except for the minor difference in the orientation of some fibres of the deep sinospiral layer, resembles so closely that of the avian ventricles that the same description of the fibre disposition of the various muscle layers as given for the ventricles of the domestic fowl is equally applicable to all the mammalian ventricles examined in the present investigation. Figures 27 - 37 are the photographs and schematic drawings of the various muscle layers of the sheep's ventricles which are presented here as the representatives of the mammalian ventricles.

The cardiac skeleton to which the various ventricular muscle layers are attached, is likewise very similar in many respects to that of the avian heart. The cartilaginous element, however, is greater in the cardiac skeleton of the mammal than in that of the birds examined.

In the mammalian ventricles the deeper cranial fibres of the deep sinospiral layer which take origin from the trigonum dextrum and the adjacent part of the left annulus are almost transversely arranged (Fig.33) and constitute a closely aggregated bundle. This bundle, instead of passing through the right A.V. valve as in the bird, courses forward in the right ventricular wall encircling the right A.V. orifice

Fig.27. Photograph of the sternocostal surface of the sheep heart showing the fibre arrangement of the superficial muscle layers of the ventricles.

X approx. $\frac{3}{4}$

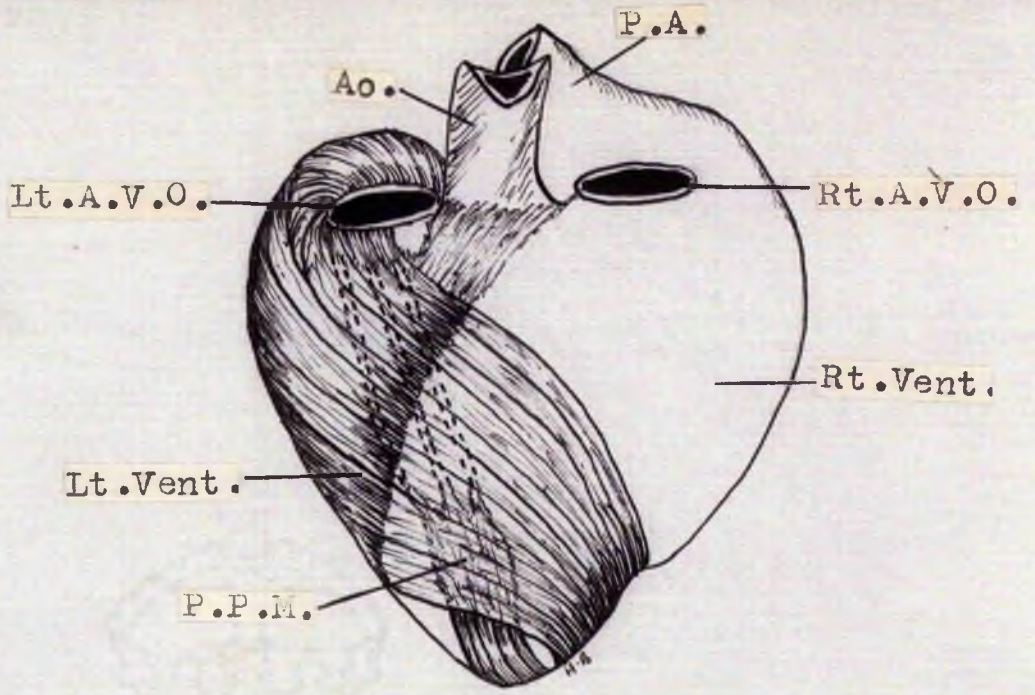

Fig.28. A schematic representation of the complete course of the superficial bulbospiral layer of the sheep heart. (Dorsal view)

X approx. normal size

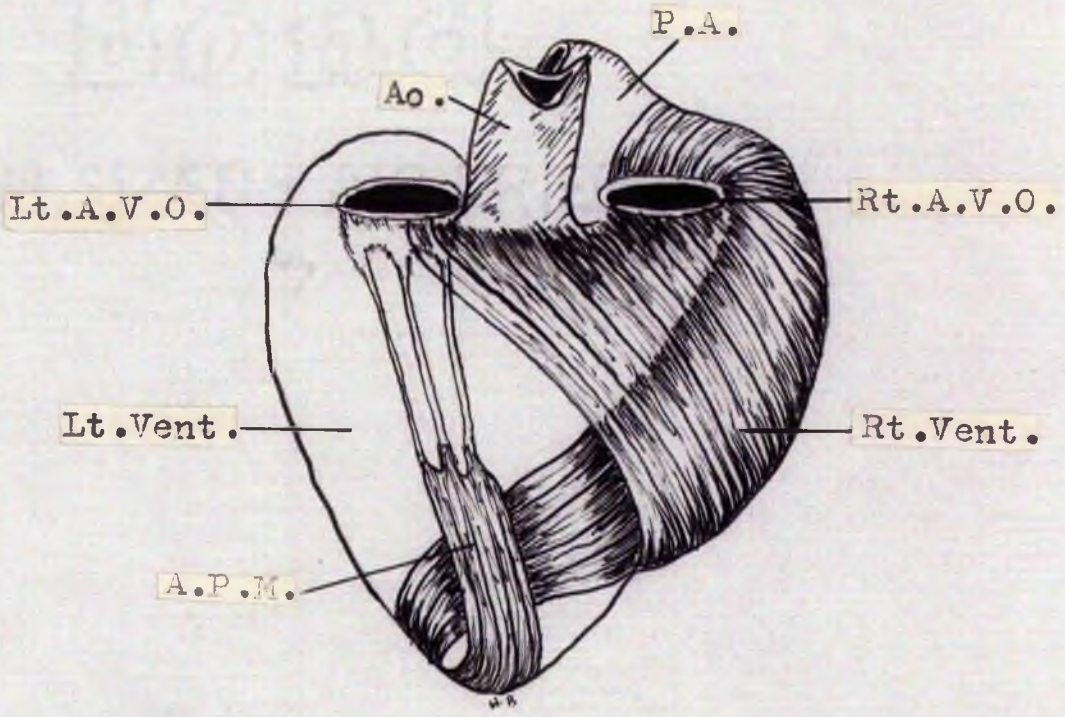

Fig.29. A schematic representation of the complete course of the superficial sinospiral layer of the sheep heart. (Dorsal view)

X approx. normal size

and terminates ventrally in the conus tendon and also into the right side of the aortic root. This transverse bundle of fibres of the deep sinospiral layer, when viewed from the interior of the right ventricle may clearly be seen even with the endocardium in situ, as a prominent slightly raised structure around the right A.V. opening (Fig.34). This bundle of fibres of the deep sinospiral layer forms the posterior crus of the Infundibulo-ventricular crest. It may be mentioned in this connection that unlike the avian right A.V. orifice which is guarded by a single muscular cusp, the corresponding opening in the mammal is guarded by a tricuspid valve; the cusps of this tricuspid valve contain only a few muscle fibres which are confined approximately in the basal one fourth of the cusps. These muscle fibres are the continuation of the atrial musculature into the cusps of the tricuspid valve and no ventricular muscle fibres are found to enter them.

The caudal fibres of the deep sinospiral layer which originate mostly from the left annulus fibrosus pass obliquely along with the remainder of the fibres of this layer in an anticlockwise direction and, after winding round the right border of the heart sink into the ventral interventricular groove as deeply as the endocardium. Having reached this plane they turn backwards on themselves in the ventral wall of the right ventricle (Fig.34) and ascending towards the ventricular base finally end in the conus tendon. In the terminal part of their course they interdigitate with the transverse fibres (posterior crus of the Infundibulo-ventricular crest) already mentioned and, these

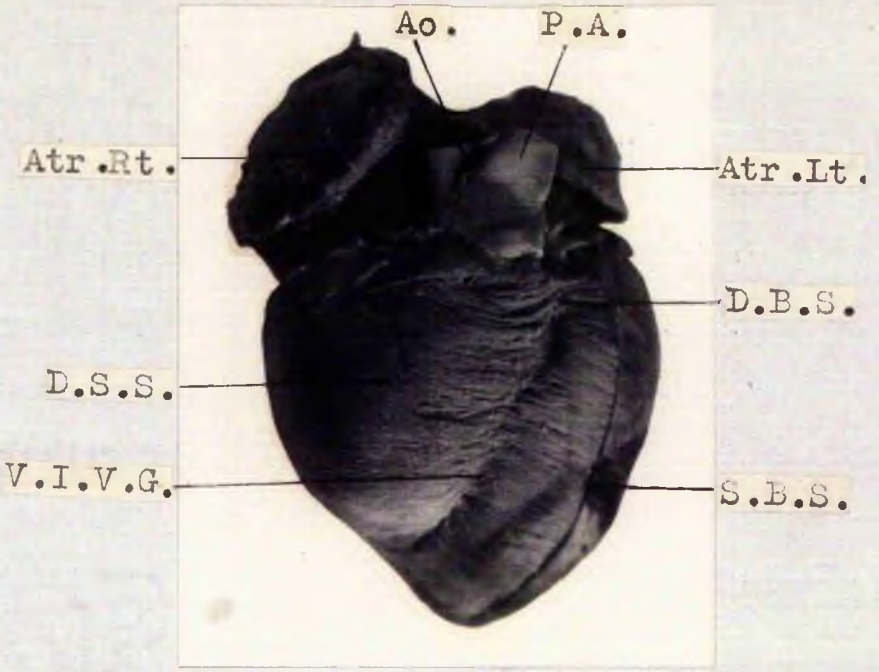

Fig.30. Photograph of the sternocostal surface of the sheep ventricles after removal of the superficial layers. Note the loop-like arrangement of the deeper fibres over the infundibulum.

X approx. ¾

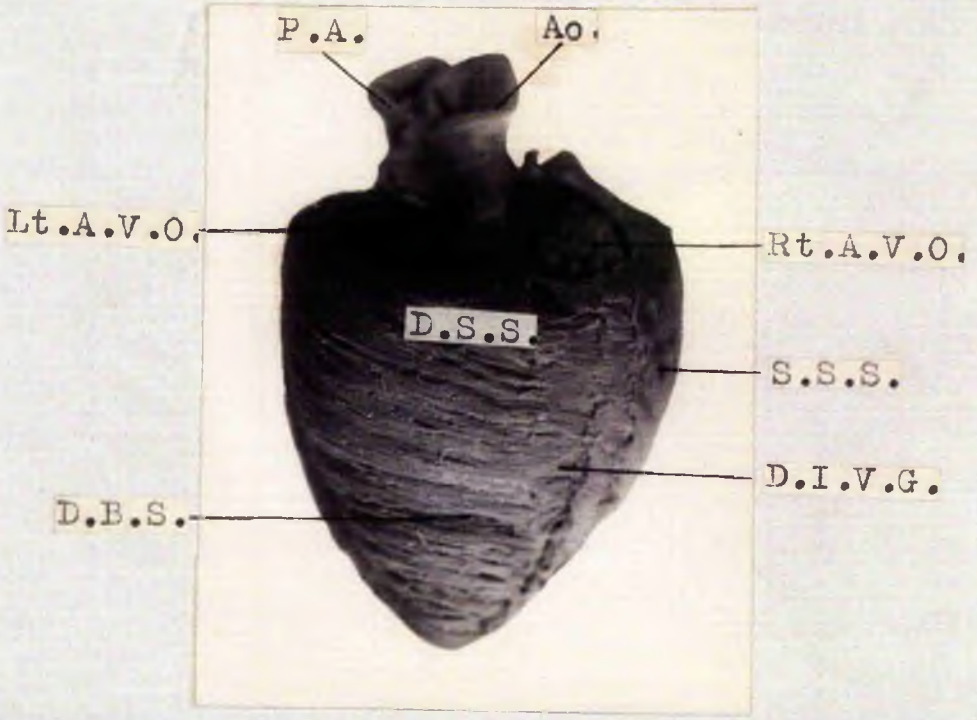

Fig.31. Photograph of the diaphragmatic surface of the sheep ventricles after reflection of the superficial layers. Note that the fibres of the deep bulbospiral layer cross the dorsal I.V. groove to pass to the right ventricle.

X approx. ¾

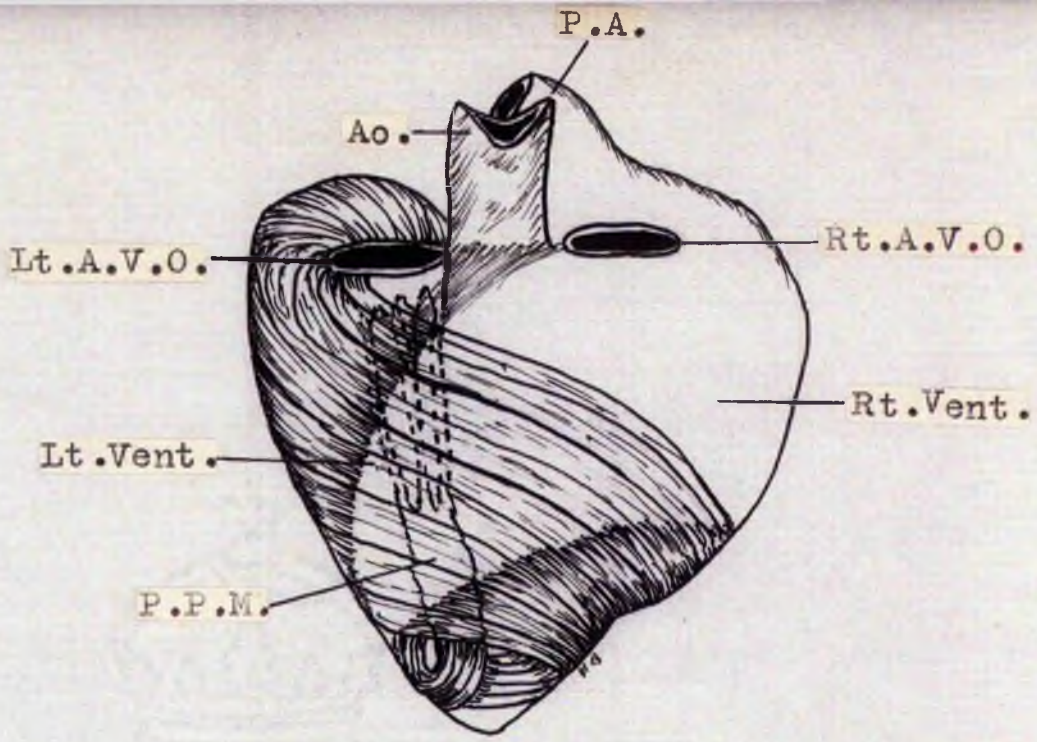

Fig.32. **Diagrammatic representation of the complete course of the deep bulbospiral layer of the sheep heart. Note that this layer passes over both ventricles.** (Dorsal view)

X approx. normal size

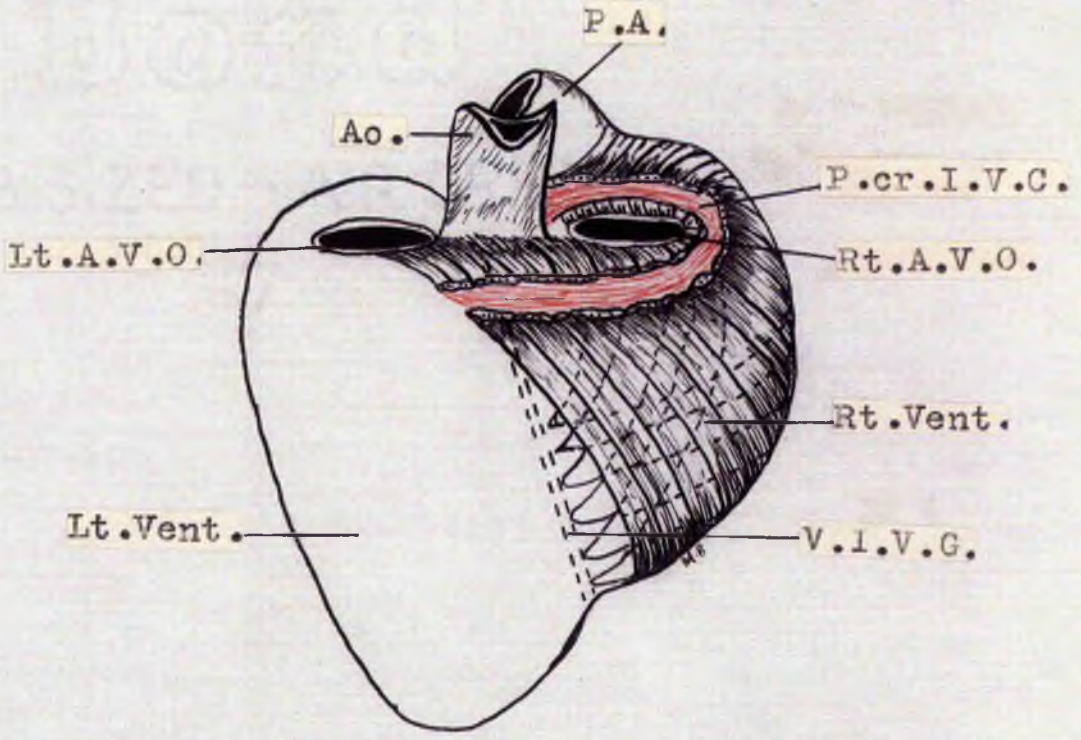

Fig.33. **Diagrammatic representation of the complete course of the deep sinospiral layer. Note that the layer is mostly limited to the right ventricle. Fibres which form the posterior crus of the I.V. crest are shown in red.** (Dorsal view)

X approx. normal size

together constitute the body of the Infundibulo-ventricular crest (Fig.34); this prominent structure lies between the inflow and the outflow tracts of the right ventricle. Where this bundle of oblique fibres has a superficial position in the ventricular wall it is not readily recognisable as a distinct entity but when it has gained a deep position and viewed from the interior, its ascending part is clearly seen as a definite band. This ascending part of the bundle constitutes the anterior crus of the Infundibulo-ventricular crest (Fig.34).

The remainder of the fibres of the deep sinospiral layer of the mammalian ventricles are orientated in exactly the same manner as in the avian ventricles. The muscle fibres which gain the deep surface of the wall of the right ventricle through the caudal region of the ventral interventricular groove eventually form the dorsal papillary muscle of the right ventricle, whereas those fibres which pass deeply close to the side of the anterior crus of the Infundibulo-ventricular crest form the ventral papillary muscle of that ventricle. It is of interest that these structures are either very rudimentary or altogether absent in the right ventricle of the bird.

Before embarking on the discussion, it is considered appropriate to summarise the special muscular features of the ventricles of birds and mammals - it is only in these two classes that the septation of the ventricles is complete.

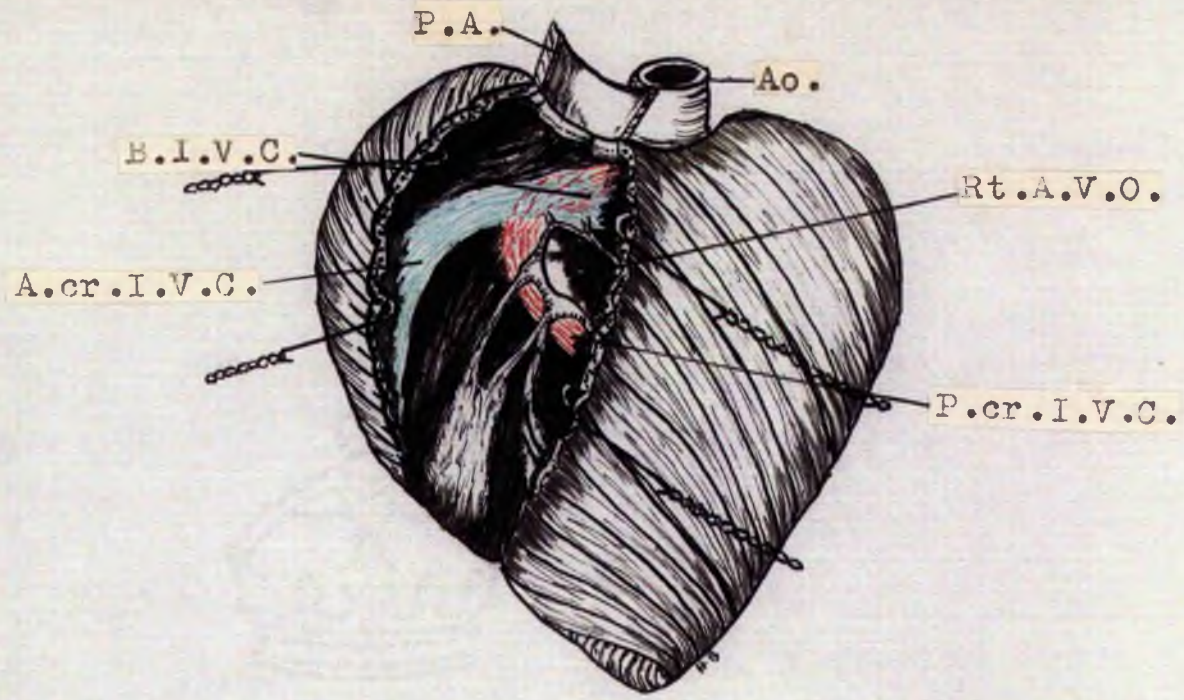

Fig.34. Drawing of the I.V. crest of the sheep heart as seen when viewed from the interior of the right ventricle. The anterior crus is shown in green and the posterior crus in red.

X approx. $\frac{3}{4}$

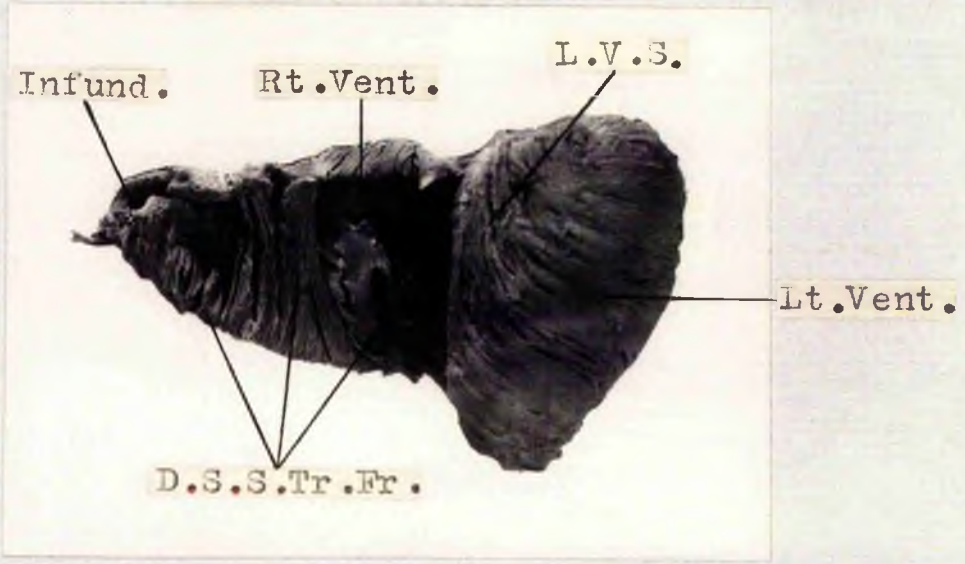

Fig.35. Photograph of the fibre arrangement of the longitudinal layer of the ventricular septum. The right ventricle is unrolled away from the left ventricle along the ventral I.V. groove.

X approx. $\frac{1}{2}$

Left ventricle

(i) The wall of the left ventricle has always been found in normal hearts to be thicker than that of the right ventricle. The left ventricular muscle fibres, except for those of its circular layer, form an open figure of eight spiral.

(ii) The vortex is confined to the left ventricle.

(iii) The apex is covered only by the superficial muscle layers whereas the deeper layers enter the substance of the ventricular wall slightly more cranially.

(iv) The circular layer of the left ventricle does not have its counterpart in the right ventricle.

(v) The superficial muscle fibres over the left ventricle are comparatively more longitudinal in disposition than over the right.

(vi) The papillary muscles in the fowl's left ventricle are rudimentary and small.

Right Ventricle

(i) The wall of the right ventricle in normal hearts is thinner than that of the left. The deeper muscle fibres provide only a segment of a cone in their course over the main part of the right ventricle and are arranged in semicircular loops over the infundibulum.

(ii) There is no vortex in this ventricle.

(iii) The apical area of the right ventricle is covered with both superficial and deep layers of muscle.

(iv) The superficial muscle fibres of this ventricle are relatively less longitudinal than those of the left ventricle.

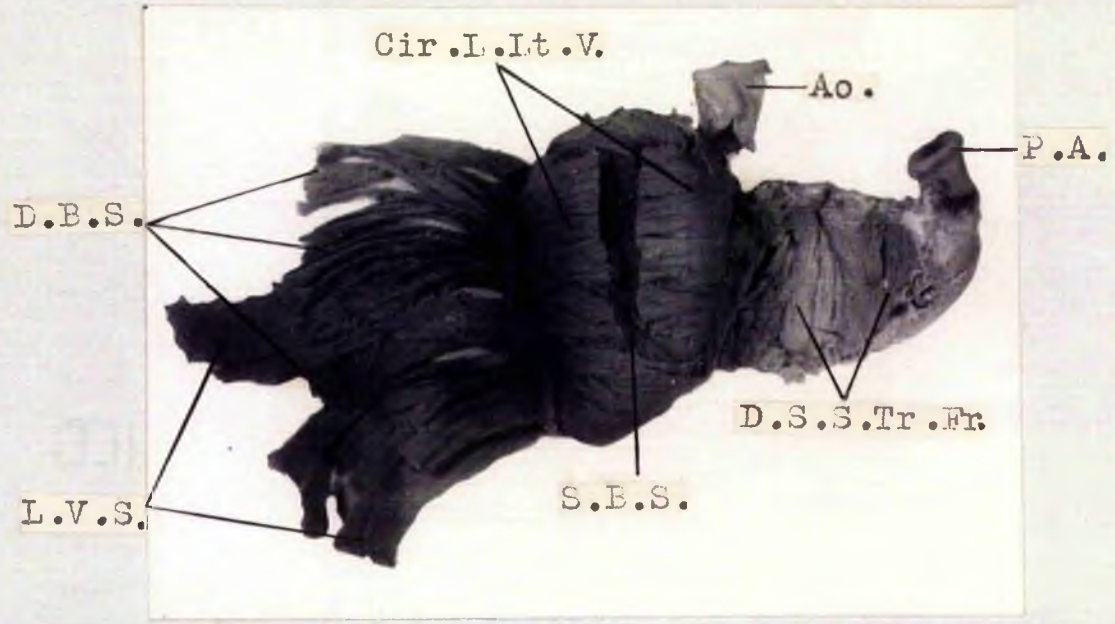

Fig. 36. Photograph of the circular layer of the left ventricle of the sheep. Note that the fibres of the other layers pass deep to this layer to gain the inner surface of the left ventricle.

X approx. ½

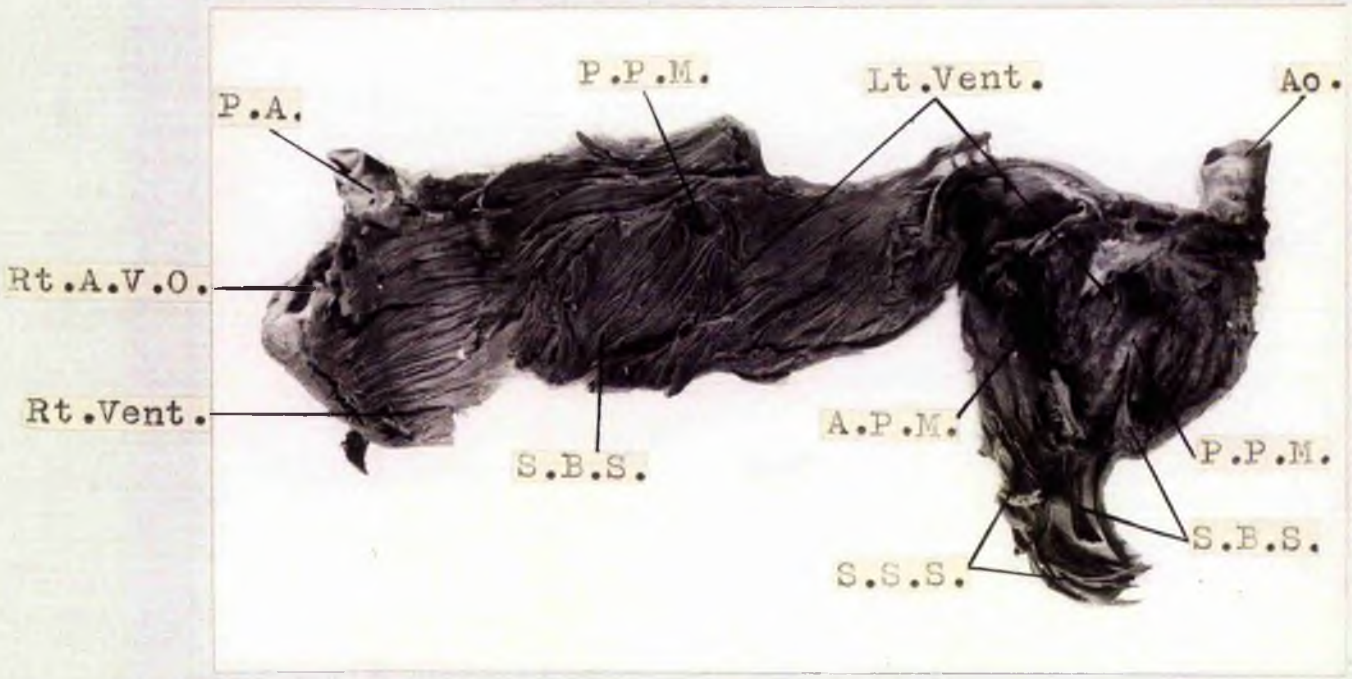

Fig.37. Photograph of the wall of the left ventricle of the sheep which is opened out as a sheet of muscle after severing its circular layer. Note the continuity of the superficial fibres with the innermost fibres of the ventricular wall.

X approx. ½

(v) The muscular valve which guards the right A.V. opening is a special feature of the avian heart.

(vi) The papillary muscles of the right ventricle of the bird are either very rudimentary or altogether absent.

DISCUSSION

DISCUSSION

It is generally considered that the mammalian ventricles are made up of a number of muscle layers (Gerdy, 1823; Pettigrew, 1864; MacCallum, 1900; Mall, 1911; Walmsley, 1929; Walmsley, 1938; and Robb and Robb, 1938, 1942). This concept has, however, been considerably modified by Lev and Simkins (1949) who have stressed the syncytial nature of the cardiac musculature, although this feature of the myocardium has been recognised by practically all the workers on the subject. Lev and Simkins, however, deny the presence of multiple layers as many investigators have found in the mammalian heart and have also been described by Pettigrew (1864) and Shaner (1923) as occurring in the bird . Lev and Simkins consider that there are only three groups of muscle fasciculi, which they have termed epicardial, middle and endocardial, and they are not separated by any connective tissue planes into laminae.

The result of the present comparative study indicates that the ventricular muscle fibres are arranged in distinct layers especially in the higher reptiles, birds and mammals. In these animals the layers are well defined and can be separated from one another by ordinary dissection although in the lower vertebrates the laminar arrangement is also present and could be seen in the stained cleared specimens, the layers are not separable as there is a considerable mingling of the superficial and deeper fibres. The fibre arrangement gets more organised and the lamination becomes better defined as the animal scale

is ascended. These ventricular muscle layers are connected with one another by a number of both gross and microscopic branches and from this point of view, it is true that the whole ventricular musculature is one syncytium. Nevertheless, groups of muscle fasciculi traverse in some particular direction in the ventricular wall and they can be separated from other groups by ordinary dissection even though there is no distinct connective tissue cleavage plane between them. These facts have also been pointed out by Mall (1911). Robb et al., (1935) and Robb and Robb (1938, 1942) have shown both by dissection and by experimental means that the separate ventricular muscle layers perform separate functions; Robb and Robb (1938) consider, for instance that the fixation of the A.V. valves during ventricular systole is due to the action of the superficial muscle layers. They furthermore, stated that various chronic heart diseases lead to differential hypertrophy of separate layers or part of them such as hypertrophy of the deep bulbospiral layer in hypertension.

Having considered these views and noted in my own specimens a definite laminar arrangement of the ventricular muscle fibres and also their dissectable nature especially in the higher vertebrates, the writer believes it reasonable to consider that the cardiac ventricles are morphologically composed of a number of muscle layers although histologically the cardiac musculature is one syncytium.

Amongst adult vertebrates the bulbus cordis is present only in the elasmobranch and the amphibian hearts. In the Teleost, the embryonic bulbus cordis is probably suppressed or undergoes degeneration and in

the adult it is both functionally and morphologically replaced by the bulbus arteriosus; this definite and very obvious entity, in all probability develops from the backward growth of the truncus arteriosus (Goodrich, 1930). If this assumption is true the bulbar part of the cardiovascular system which lies between the ventricle and the beginning of the ventral aorta in Teleosts, should not therefore be called the bulbus cordis although many workers continue to do so (Russell Brook, 1956); such a supposition is supported by the fact that the region in question does not contain cardiac muscle. In the reptile septa appear in the embryonic bulbus cordis which thereafter participates in the formation of the origin of three great vessels emerging from the ventricle. In birds and mammals the bulbus is divided into a right and a left part by the aortic septum. The right part is absorbed into the right ventricle and forms the infundibulum while the left part does not grow correspondingly and certainly cannot be recognised as a definite entity in the adult heart.

The general arrangement of the musculature of the ventricles and the bulbus cordis of the vertebrate heart , as observed in the present investigation, is mostly confirmatory to the findings of various previous workers.

No superficial sling-like muscle layer as described by Pettigrew (1864) in the ventricle of salmon or by Shaner (1923) in the ventricle of sturgeon, is present in the ventricle of cod and haddock. Fig.4 is a photograph of a transverse section of the haddock ventricle which clearly shows that the circular layer is lying immediately underneath

the epicardium and that no other layer is situated between the two.

The muscular architecture of the frog's ventricle as seen in this investigation, is almost exactly like that described by Shaner (1923) except that the inner trabeculae are not arranged in two sheets placed along the ventral and dorsal margins of the A.V. orifice as stated by him; they are arranged all around the A.V. opening and are attached to the caudal margin of the A.V. funnel and A.V. valve. A similar arrangement of the trabeculae is also described by Davies and Francis (1941) in the ventricle of the salamander (S. salamandra).

The present findings support the view of Shaner (1923) that the spiral arrangement of the ventricular muscle fibres first appears in the reptilian heart. The lizard's ventricular architecture is very much like that of the frog except the circular fibres show a certain degree of spiral orientation. In the grass snake, this spiral disposition is more marked. In the alligator (A. mississipiens), it is almost similar to that found in the bird and the mammal (Shaner, 1924).

The superficial longitudinal layer as found in the ophidian ventricle(s) was not seen in the ventricle in the lower reptile examined, namely the green lizard. In the tortoise (Chelonia) ventricle, Shaner (1923) described the definite spiral arrangement of the circular fibres but mentioned no superficial longitudinal layer although he (Shaner, 1924) found a fairly thick longitudinal layer in the alligator's ventricles. In the grass snake this layer is distinct and dissectable. This layer is definitely absent in the ventricle of the frog, cod and haddock. Thus it appears from the present comparative study that as

animals ascend in the evolutionary scale three distinct changes occur in the ventricular muscle, viz:-

(i) The fibres lose the haphasard orientation and become better organised, and

(ii) A laminar arrangement becomes more and more evident and more easily demonstrable.

(iii) The circular disposition of the mass of fibres is gradually replaced by a spiral arrangement.

In all the ophidian hearts examined, a very characteristic feature has been the great relative thickness of the ventral wall of the ventricle as compared with the dorsal wall, the ratio being approximately 3 : 1. There appears to be no mention of this remarkable feature in the literature and the candidate postulates that it may be associated with the fact that in progression, the snake moves with its ventral body wall apposed to the ground; furthermore in this connexion it is of interest to correlate the absence of sterna in snakes. The correlation of heart form with other physical factors is, however, recognised to be a wide problem but the candidate intends to make a full study of the snake's heart in the hope that it may produce further evidence to account for the great discrepancy that exists in the relative thickness of the two walls of the ventricle.

The candidate's findings in the avian and mammalian ventricles are very similar to those of previous workers except for a few minor differences. Mall (1911), in the human heart, described two vortices, one for the right and the other for the left ventricle. The present

series of mammalian and avian hearts, however, showed only one vortex confined to the left ventricle. Weber (1831) stated that the vortex is limited to the left ventricle only and the superficial fibres of the right ventricle do not form a vortex. Pettigrew (1864) was of the same opinion, when he stated that the external fibres of the right ventricle enter the entire anterior coronary groove along its length to become internal and not at a limited site as the apex of the left ventricle.

The longitudinal layer of the ventricular septum has long been known to the anatomists and was fully described by MacCallum (1900) in pig, Mall (1911) in human and Shaner (1923) in domestic fowl. Various names have been given to this layer by different workers. Mall (1911), Shaner (1923) and Flett (1928) called it "longitudinal layer of the right ventricle". Gerdy (1823), MacCallum (1900) and Walmsley (1929) called it as "interventricular layer". Nevertheless, it appears that neither of these names conveys a clear idea as regards the position of this layer in the ventricular wall. Although the fasciculi of this layer show various degrees of obliquity the layer as a whole tends to pass along the long axis of the septum and it is therefore considered that the name longitudinal layer of the ventricular septum is appropriate.

The origin of the longitudinal layer of the ventricular septum, certainly in the ox, sheep and pig, is more extensive than that described by Mall (1911) in the human heart. He limited the origin to the aortic root only. In the present investigation, both in mammals and birds,

the layer was found to be attached to the aortic root and trigonum dextrum. This agrees with the findings of Flett (1928) in the human heart. Furthermore, this layer, after emerging on the surface of the left ventricle blends with the deep bulbospiral layer and also with those fibres of the deep sinospiral layer which cross the ventral interventricular grooves rather than with the sinospiral layer alone as described by Mall (1911).

In the human heart Brandt (1953) implies that the Infundibulo-ventricular crest is a separate muscle, having an origin in the conus tendon and an insertion into the lower part of the ventricular septum. However, it appears from the present observations in the other mammals that the muscle of the crest is not a separate entity but represents only a part of the deep sinospiral layer. Fibres of this portion of the deep sinospiral layer are attached to the trigonum dextrum and the adjacent part of the left annulus fibrosus and pass ventrally in two bundles, which reunite in the region of the conus tendon and there form the body of the crest. One bundle passing transversely round the right A.V. opening comes to constitute the posterior crus of the crest. The other bundle passes spirally around the right ventricle and penetrating the lower part of the ventral interventricular groove ascends on the endocardial surface of the ventral wall of that ventricle and finally reaches the conus tendon; while this bundle is on the external surface of the heart it is not readily distinguishable from the rest of the layer. The ascending part of the bundle which, however, is seen as a slightly raised band when viewed from the inner surface of

the right ventricle, constitutes the anterior crus of the crest. The two crura join each other close to the comus-tendon and the fibres of the one interdigitating with those of the other at an acute angle to form the body of the crest (Fig.34). The first of these bundles which has been described namely that one which passes transversely round the right A.V. orifice is a prominent structure when viewed from the interior of the right ventricle after the requisite dissection, furthermore when its presence is appreciated it may even be recognised before the endocardium is disturbed. It is postulated that this bundle of fibres, on account of its position and orientation, may be associated with a diminution of the diameter of the right A.V. orifice during ventricular systole. Some supporting evidence for such an implied functional arrangement will be given in the following paragraph during the discussion of the corresponding group of muscle fibres in the bird.

The muscle fibres of the right A.V. valve in the bird are of special interest. They belong to the deep sinospiral layer which describes a course that is similar to the fibres of the posterior crus of the mammalian Infundibulo-ventricular crest. In the intermediate part of its course this group of fibres lies within the single cusp of the right A.V. valve. In so doing these fibres, through their valvular relationship, constitute a mechanism for the complete closure of the right A.V. orifice whereas in the mammal the corresponding fibres as has already been postulated, assist in the closure by narrowing the right A.V. orifice. The two structures on account of

their muscular relationship may, therefore, be regarded as homologous, but they are not strictly analogous on account of the difference in the mechanism in the right A.V. valves of birds and mammals. As far as I am aware this comparison of the avian and mammalian right A.V. region has not previously been described.

Although, in the past, there was divergence of opinion regarding the presence of muscle fibres in the A.V. valves of the normal mammalian heart, it is now generally agreed that the atrial muscle fibres extend for a small distance into the A.V. valves of the mammal including man (Walmsley, 1929 and Dow and Harper, 1932). The presence of atrial muscle fibres in the basal one fourth of the cusps of the mammalian tricuspid valve, as found in this investigation, therefore, entirely agrees with the findings of the other workers.

The deep bulbospiral layer as described in this investigation is quite different from the muscle layer which Mall (1911), Shaner (1923) and Flett (1928) described by that name. The deep bulbospiral layer according to them, is limited to the left ventricle only and is therefore equivalent to the circular layer of the left ventricle of the present investigation. It appears from their description that no muscle layer is situated between the superficial bulbospiral layer and the circular layer of the left ventricle (their deep bulbospiral layer), although in all mammalian and avian hearts, I have found that a definite, although relatively thin, layer is situated between them. This layer is attached to an area of the cardiac skeleton, corresponding to the origin of the superficial bulbospiral layer but in a deeper

plane and it is not limited to the left ventricle only. Figs.31 and 32 show that the fibres of the deep layer which take their origin from the ventral portion of the cardiac skeleton are crossing the dorsal interventricular groove and thus prove their biventricular nature. Fig.30 shows the origin of this layer from the conus tendon and the pulmonary root thereby indicating that the layer is not confined to the left ventricle. It is this layer, which I have described as the deep bulbospiral layer whereas the layer which lies deep to it and is confined to the left ventricle only (Fig.36) I have designated the circular layer of the left ventricle. The deep bulbospiral layer as described in the present investigation, is equivalent to the combined second and third layers of Pettigrew (1864) and the circular layer of the left ventricle is the same as his fourth or central layer. Shaner (1928) in his review of the adult structure of the pig's ventricles stated, "Beneath the outer vortex layer lies the complex middle layer. The more superficial fibres of the layer surround both the ventricles. The deeper fibres form a cylinder in the left ventricle which fits over the papillary muscles and trabeculae carnae. The superficial fibres of the middle layer, on both surfaces of the heart, jump across interventricular grooves. Above they are attached to the heart base, below they merge with the vortex layer at the apex. In the left ventricle, the middle layer is much thicker and it is to the deeper fibres of the middle layer, which belongs wholly to the left ventricle that Mall applied the term 'deep bulbospiral layer'." These observations of Shaner in the pig entirely agree with the present

findings in the birds and mammals. The deep bulbospiral and deep sinospiral layers of the present investigation together form the superficial stratum of his middle layer and the circular layer of the left ventricle as the deep stratum of his middle layer. Robb and Robb (1942) admitted the presence of a deep layer in the left ventricular wall in addition to their deep bulbospiral layer. They stated that the fibres of this deep layer originate from 'the anterior curve of the left A.V. ring' but they described this deep layer as a part of the deep sinospiral layer, although, according to them, the superficial bulbospiral layer also arises from this same area but in a superficial plane.

Complete separation of the systemic and pulmonary circulations and a definite well marked spiral arrangement of the ventricular muscle fibres are found only in the bird and the mammal - the two classes of warm blooded animals. Furthermore, the blood pressure in these warm blooded animals is higher than that in the cold blooded ones. The following table shows the average systolic blood pressure in some of the vertebrates:-

Man (Adult)	130 mm. of Hg. (Spector, 1956)
Cattle	134 mm. of Hg. (Spector, 1956)
Albino rat	116 mm. of Hg. (Spector, 1956)
Domestic fowl	150 mm. of Hg. (Spector, 1956)
Lizard	40 mm. of Hg. (Clark, 1927)
Snake, ring	89 mm. of Hg. (Spector, 1956)
Frog	43 mm. of Hg. (Spector, 1956)
Dog fish	32 mm. of Hg. (Spector, 1956)

It is generally conceded that there are several factors concerned with the maintenance of blood pressure and among these, one of the most important is the pumping power of the ventricles. The ventricles of the warm blooded animals, therefore, must be relatively more powerful than those of the cold blooded, in order to maintain their higher blood pressure. The spiral arrangement of the ventricular muscle fibres in the warm blooded animal makes its heart a more powerful pumping organ than that of the cold blooded one. It is postulated that the spiral arrangement of the ventricular muscle fibres results in the blood being "wrung out" of the ventricles rather than being "squeesed"out" as occurs in ventricles which have only circularly arranged muscle fibres.

The spiral arrangement of the ventricular muscle fibres in birds and mammals is probably one of the many factors which helps to maintain homoiothermia in them. This arrangement first appears in a class of vertebrates viz. the reptiles, which though classified as poikilothermal, maintain the body temperature better than the other classes of the cold blooded animals, viz. the fish and the amphibia (Clark 1927). This morphological change in the arrangement of the ventricular muscle fibres and separation of the two circulations thus seem to have evolved simultaneously with the ability of the animal to maintain its body temperature.

A higher rate of metabolism is essential in the homoiothermal animal to keep its high body temperature. The average B.M.R. in the warm blooded animals is from 900 - 1,000 Calories per sq. metre of the

body surface whereas that of the cold blooded animals is far below 100 Calories per sq. metre of the body surface (Clark 1927, Spector 1956). A higher metabolic rate, in turn, requires oxygen and food materials to reach the tissues rapidly and in sufficient quantities. This is achieved in the higher vertebrates, along with other factors, by the spiral arrangement of the ventricular muscle fibres and by the complete separation of the systemic and pulmonary circulations. The spiral orientation of the muscle fibres helps to increase the cardiac output by augmenting the pumping power of the ventricles and thereby increases the supply of oxygen and food materials to the tissues, while the separation of the circulations allows only the oxygenated blood to reach the tissues.

Therefore, it seems reasonable to postulate that the spiral disposition of the ventricular musculature in the higher vertebrates has evolved in response to the mechanical requirement and, also indirectly, to the thermal need of the animal.

SUMMARY

SUMMARY

(1) A number of hearts of various representative vertebrate animals have been studied to observe the arrangement of the ventricular muscle fibres. Larger hearts were boiled in acidulated water and subsequently treated with 90% alcohol before dissection. Smaller hearts were treated with 90% alcohol only. The latter group was also stained with 0.05% Benzoquinone and cleared in Oil of Wintergreen and the muscle fibres traced under the binocular microscope. Paraffin sections of smaller hearts were also examined as an adjunct.

(2) Histologically, the whole of the ventricular muscle constitutes a syncytium. Nevertheless, groups of muscle fibres are so orientated that they form lamina which in the larger hearts, and particularly so in Mammals, may clearly be displayed by ordinary dissection.

(3) Mammalian and avian ventricles are made up of spirally arranged muscle layers and they are classified into two groups - the "Bulbospiral group" and the "Sinospiral group". Ichthyopsidan ventricles consist of an outer thin circular layer and dense inner trabeculae. The ophidian ventricle(s) has a superficial longitudinal layer, a deep circular layer and a mass of inner trabeculae.

(4) The deep bulbospiral layer of the present investigation is different from that described by the previous investigators. The circular layer of the left ventricle of the present work is the same as the deep bulbospiral layer of other workers.

(5) The spiral arrangement of the ventricular muscle fibres first appears in the lower reptiles and is most apparent in the ventricles of birds and mammals.

(6) An attempt has been made to correlate the function with the evolutionary changes in the muscular architecture of the ventricles. It has been suggested and discussed that the spiral arrangement of the ventricular muscle fibres in the higher vertebrates has evolved in response to mechanical requirements and also indirectly to thermal need of the homoiothermal animals.

PART II

INNERVATION OF THE BULBUS CORDIS AND THE VENTRICLES OF THE VERTEBRATE HEART

A BRIEF REVIEW OF THE LITERATURE ON THE VENTRICULAR INNERVATION

A BRIEF REVIEW OF THE LITERATURE ON THE VENTRICULAR INNERVATION

The literature on the ventricular innervation dates back to the later part of the eighteenth century when Scarpa (1794) first described the presence of ganglion cells on the epicardial nerves of the ventricles of mammals. Since then the innervation of the heart has drawn the attention of numerous workers. Lee (1849) observed with the naked eye, ganglion cells on the epicardial nerves of the ventricles of man, ox and heifer. Berkley (1894) using a silver technique recorded two types of nerve endings in the ventricles - a simple knob type and a complex pennate formation. Mollard (1908) found nerve cells in the ventricular wall of mammals, reptiles and amphibia. Wilson (1909) recorded a rich plexus of nerves and groups of ganglion cells in the A.V. bundle of calf, sheep and pig.

The next important observation was made by Meikeljohn (1913, 1914). She noted a considerable difference in the degree of innervation of the conducting system of the different species of mammals. She also studied the topography of the cardiac ganglion cells in the rat and found that the ventricles were devoid of nerve cells. Holmes (1921) noted bundles of nerve fibres and groups of nerve cells in the A.V. bundle of mammals and further observed a relatively greater abundance of nerves in the ox A.V. bundle than in that of the other animals studied by him. Perman (1924) recorded the presence of nerve cells in the ventricles of calf, sheep, goat and guinea pig and according to him the cells were confined to the region close to the A.V. sulcus.

Woollard (1926) made a detailed study of the intrinsic innervation of the heart. He found no ganglion cell in the mammalian ventricle. He did, however, find ganglia in the ophidian ventricle. According to him the cardiac nerve cells in the atria are subepicardial in position and parasympathetic in character. He, furthermore, observed that the ventricles are predominantly, if not solely, supplied by the sympathetic nerves. As regards the nerve endings, he found pericellular and pericapsular preganglionic endings, intraprotoplasmic endings in the muscle cells and simple knob and brush-like sensory endings in the epicardium and endocardium. In his opinion, cappilaries are devoid of nerves. Blackhall-Morison (1926) described two types of nerve endings in the heart - the vagal type in the form of "contact bulbs" and the sympathetic type in the form of "branched endings". Jones (1927), in the human heart, observed intracellular endings with a neuro-myofribillar continuity. He also noticed isolated nerve cells in all parts of the ventricular wall.

Boeke (1932) observed that a network of fine nerve fibres surrounds the muscle elements and no individual nerve endings are present. He called the network the "sympathetic ground plexus". In the tortoise and sheep, however, he found that fine branches from the ground plexus penetrated the muscle cells and formed intraprotoplasmic endings with a periterminal network. In 1949, Boeke gave an account of his concept of the terminations of the autonomic nerves. He believes that the autonomic end-apparatus is built up of a reticulum of anastomosing "interstitial cells" and that the neuro-fibrillae of both

the sympathetic and parasympathetic postganglionic nerves traverse it. He further believes that the interstitial cells form synaptic connections between the nerve fibres and the innervated structures.

In the opinion of Stöhr (1932, 1934, 1935 and 1957) the autonomic end-formation in the cardiac ventricles as well as in all other vegetatively innervated organs is composed of a neuro-fibrillar network which he designated "terminal reticulum"; the fibrils of this network penetrate into the protoplasm of the innervated cells. This network is associated with the interstitial cells and Schwann cells. Apart from the terminal reticulum he has described various types of afferent endings in the heart. Reiser (1934) also described the same type of network. He called the nucleated part of the network - "preterminal reticulum" and the non-nucleated part the "terminal reticulum".

Blair and Davies (1935), like Meiklejohn, noted a species difference in the innervation of the conducting system and also recorded intraprotoplasmic endings in the muscle cells of the media of the coronary arteries of ox but could not find the same in man. Nonidez (1936, 1937, 1939, 1941, 1943 and 1944), a strong critic of the theory of a terminal network, has found bulbs, loops and reticulated endings in the myocardium. He asserts that the terminal network is not a neural structure but a network of agyrophilic reticular tissue. He also commented that the ortho and parasympathetic nerve fibres could be differentially impregnated with a modified Cajal method. Other workers, however, question the reliability of this technique.

Seto (1936), using modified Bielschowsky-Gros technique, found a

nervous terminal network in the human heart. According to him, all cells associated with this network are Schwann cells. Nettleship (1936) found an extensive subendocardial network with many "nodal points". King (1939) noted different types of motor and sensory endings in the methylene blue stained rat ventricles, but he could not determine whether the endings are epi or hypolemmal in position.

In 1945 Kaylor using Nonides technique found only parasympathetic fibres in the ventricles of the guinea pig, a result exactly opposite to that of Nonides. Lavrentiev (1946) noted that in the periphery both sympathetic and parasympathetic fibres travel in the same Schwann plasmodium. He is of the opinion that all cardiac ganglion cells are parasympathetic and the ventricles are supplied only by the parasympathetic nerves.

Stotler and McMahon (1947) after studying the innervation of the human conducting system arrived at the significant conclusion that the peripheral Purkinje fibres are devoid of nerves, but they found organised endings in the proximal part of the A.V. bundle. They could detect no nerve endings in the ventricular myocardium. Akkeringa (1949) on the other hand, found that the Purkinje fibres are covered with a fine network, formed by the interlacing processes of the interstitial cells. Landau (1950) believes that the autonomic end-apparatus is a syncytial network of the interstitial cells. Toheng (1951) noted that the ventricular nerve cells are confined to an area close to the A.V. sulcus. He also found intraprotoplasmic nerve endings. While Field (1951) confirmed the species variation of the nervous complement

of the A.V. bundle in a number of mammals, he found that organised nerve endings are "conspicuously absent". Instead he reported a network of fine nerve fibres in the A.V. bundle.

Davies et al (1952) after studying the ventricular innervation in many orders of mammals, found that the ventricular nerve cells are present only in Artiodactyla and Cetacea, and that they are situated subepicardially and in relation to the A.V. bundle. They found no nerve endings in the ventricular myocardium but noted simple knob-like endings in the epicardium and endocardium and a fine plexus and terminal knob-like endings on the surface of some of the A.V. bundle muscle fibres.

Mitchell et al (1953) found subepicardial ganglion cells in the ventricles of the rhesus monkey and rabbit. In the former the ganglion cells were numerous and were found even close to the ventricular apex. They believe that the majority of these nerve cells are multipolar. Mitchell (1953 and 1956) believes in the existence of a terminal nerve network in all vegetatively innervated organs and asserts that the network is not an artefact due to formalin fixation as suggested by some investigators. He reviewed and discussed the whole problem in great detail in his book on the cardiovascular innervation (1956).

Mayling (1948, 1953) made a significant contribution to the problem of the morphology of the autonomic end-apparatus. After examining the innervation of various organs including the heart, he arrived at the conclusion that the end-formation is composed of a nervous terminal network, built up by the interlacing processes of the

interstitial cells. This network supplies all the structures of the ventricles including the capillaries.

Sato (1954), a student of Seto and working with his modified Bielschowsky method, observed the same terminal network in the canine heart. He thinks the network stands in "control over almost any kind of tissues" in all the three layers of the heart. In addition to the network he also noted tapering medullated nerve fibres.

Recently Holmes (1956 and 1957), using silver impregnation and methylene blue techniques found a "terminal nervous network" with cells in its meshes in the endocardium of the canine atria. Apart from the network, he also noted "circumscribed end formations" related to thick myelinated fibres; these are situated particularly about the points of entry of the large veins into the atria. In his opinion this second type of ending is receptor in function.

OBSERVATIONS

OBSERVATIONS

GENERAL DISTRIBUTION OF THE VENTRICULAR NERVES

The innervation of the heart is known to be derived from sympathetic and parasympathetic nerves. These intermingle on their way to the heart especially in the cardiac plexuses, and since morphological differences between them have not been as yet definitely established, they have proved indistinguishable in their course through the ventricular wall.

Before describing the morphology of the ventricular innervation it is considered appropriate to mention at this stage that the autonomic nervous system is considered here as consisting of both sensory and motor elements. There appears, however, to be considerable difference of opinion regarding the constitution of the autonomic system. At present, there are two views about this subject. According to one view this system is defined as a purely efferent one (Kuntz, 1953), as enunciated by Langley (1903). The writers who subscribe to this view admit the presence of visceral afferent fibres but they believe that these fibres do not belong to the autonomic system because they have their nerve cells in sensory ganglia on the cerebro-spinal nerves like all sensory fibres irrespective of their distribution. On account of this they regard that the autonomic system should be considered as entirely motor. According to the other view, the autonomic nervous system is considered to be comprised of both afferent and efferent components (Mitchell, 1953, 1956 and Stöhr, 1957).

As the candidate ———————————————————

personally agrees with the second view, the autonomic nerves which supply the ventricles are regarded in the present investigation, as having both afferent and efferent components.

The anatomical pattern of the innervation of the ventricles in all the animals studied in the present investigation has proved to be basically the same. There are three plexuses, a sub-epicardial, a myocardial and a sub-endocardial. These are formed by bundles of nerve fibres which are connected with a more peripheral terminal nerve network supplying the various ventricular components as will be described later. Finally it is to be noted that in general the ventricles possess intrinsic nerve cells.

In the various animals certain differences have, however, been found in the paths by which the nerves reach the ventricular plexuses as will be described in the following general account of the nerve supply of the ventricles.

In the rat (Mus norvegicus), dog (Canis familiaris) and in the domestic fowl (Gallus domesticus), the ventricular nerves travel mainly as the plexuses which surround the coronary arteries and their branches and also along the atrio-ventricular bundle of His. Only a few nerves reach the ventricles independently of these paths.

Over the surface of the ventricles, nerve bundles derived from the nerve plexuses accompanying the coronary arteries and also the nerve bundles reaching the ventricles independently of these coronary plexuses divide and subdivide, the branches joining one another to form a subepicardial plexus. Branches of this surface plexus are

mainly distributed to the ventricular wall barring most of the ventricular septum which is mainly supplied by the nerve bundles accompanying the A.V. bundle. From the surface plexus, bundles of nerves penetrate at various points into the myocardium, either alone or along with the branches of the coronary arteries where they form a secondary plexus - "the myocardial plexus" (Fig.38). Nerves also reach the ventricular myocardium by way of many large and small bundles of nerves accompanying the A.V. bundle. These form plexus both within and on the surface of the A.V. bundle. This plexus is particularly reach in the proximal part of the bundle of the rat (Fig.39), but is not so well developed in the dog and fowl, particularly the former, in the bundle of which relatively few nerve bundles are present. Many of the nerves of the A.V. bundle leave it and run for a considerable distance deep to the endocardium of the septum (Fig.40) before turning into the myocardium where they help to form the myocardial plexus.

The subepicardial, myocardial and subendocardial plexuses are considered to be composed of afferent and efferent fibres of both ortho and parasympathetic cardiac nerves. The thickly medullated fibres are regarded as afferent, and form a part of the extrinsic cardiac nerves. The apparently non-medullated fibres of the plexuses are considered to be efferent and to be derived from the extrinsic cardiac nerves as well as from the axons of the multipolar nerve cells of the intrinsic cardiac ganglia, to be described later. Branches of these plexuses ultimately supply the various components of the ventricles through the terminal

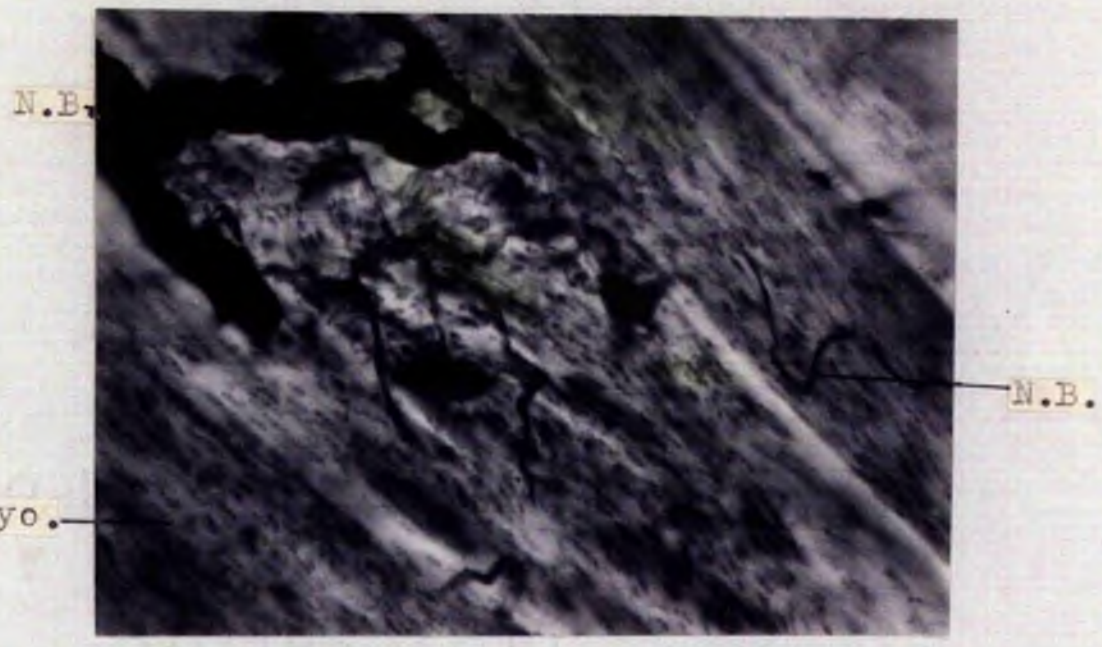

Fig.38. Sagittal section of the left ventricular wall of the domestic fowl showing the myocardial plexus formed by bigger and smaller bundles of nerve fibres.

(Gros-Schultze) X 230

Fig.39. An oblique section through the proximal part of the rat ventricles. Numerous large and small bundles of nerves are seen in the proximal part of the A.V. bundle.

(Gros-Schultze) X 90

nerve network.

In the green lizard (Lacerta viridis) and in the grass snake (Natrix natrix), nerves in large bundles reach the ventricles by passing directly from the sides of the great vessels and from the walls of the atria; only a few nerves travel along the coronary arteries. The nerves from the sides of the great vessels pass at first into the subepicardial plexus before they penetrate the myocardium. Those coming from the walls of the atria are situated superficial and deep to the musculature of the A.V. funnel (Fig.41) and surrounds the A.V. opening on their way to the ventricle. They pass straight into the myocardial plexus.

In the frog (Rana temporaria) nerves pass to the ventricle along the ventral aorta and the bulbus cordis, and enter the subepicardial plexus on their way to the myocardial plexus. The nerve bundles from the walls of the atria take a course similar to that found in the green lizard, on their way to the myocardial plexus of the ventricle (Fig.42). A few nerve fibres on their way to the ventricle also pass along the coronary arteries which are confined to the bulbus cordis.

In cod (Gadus morrhua) and haddock (Gadus aeglefinus) the writer had very little success in impregnating the nervous element of the ventricle with silver by the three methods used although a large number of hearts were investigated. Only a few scattered nerve fibres were seen. It should be mentioned in this connection that all the animals used in the present investigation with the exception of fish were killed and fixed immediately by the writer himself. But as no

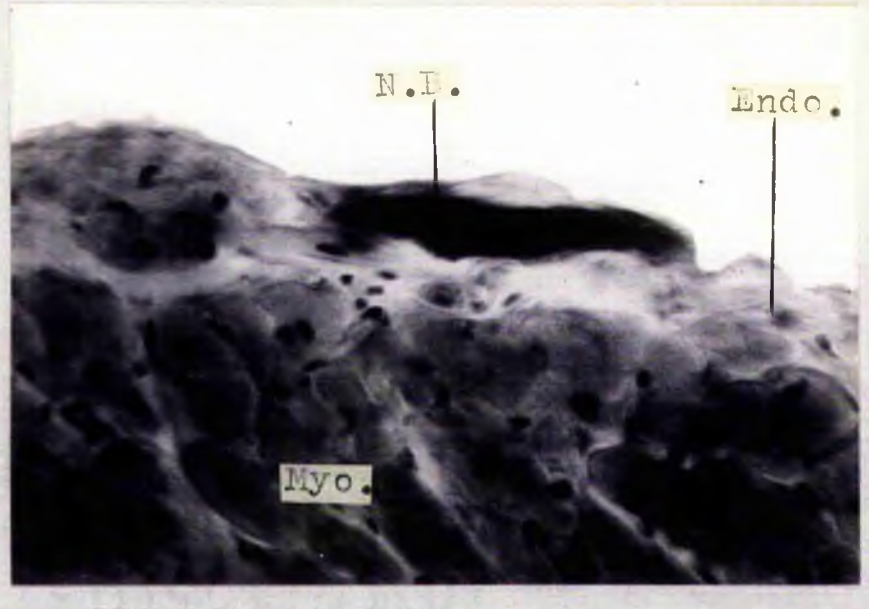

Fig.40. An oblique section of the rat heart. Shows a big bundle of nerve fibres in the subendocardium of the right surface of the ventricular septum.

(Gros-Schultze) X 350

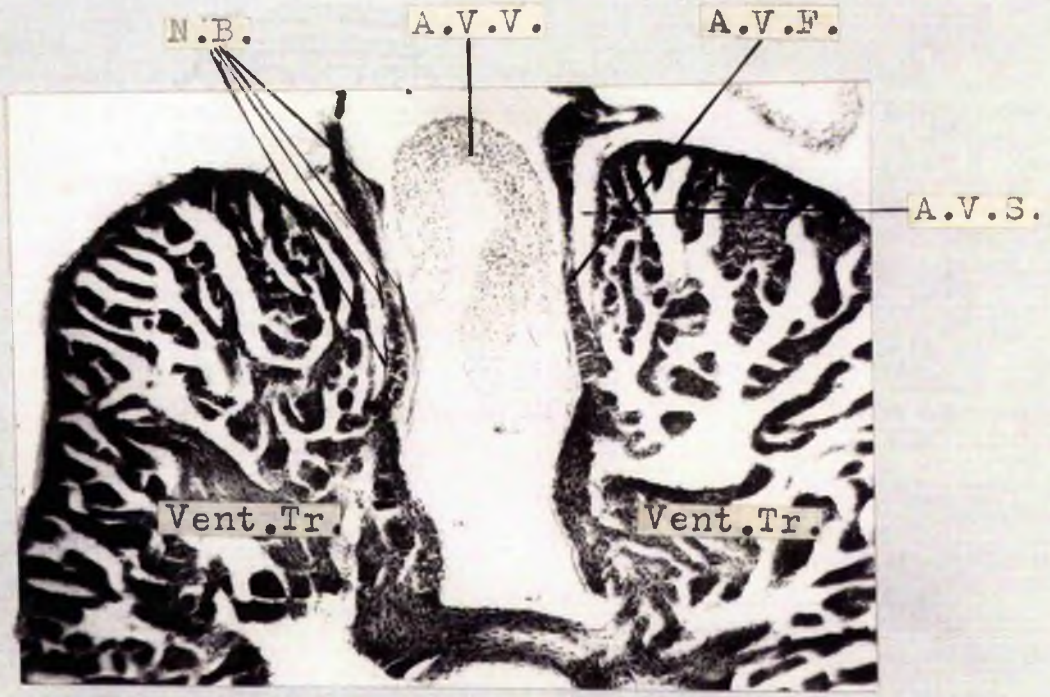

Fig.41. Sagittal section of the lizard heart. Shows that nerve bundles enter the ventricle surrounding the A.V. opening. Note that the bundles are situated both superficial and deep to the musculature of the A.V. funnel.

(Bodian) X 60

live fish were available, bottles containing fixatives were given to a fisherman, who was requested to place the hearts in the fixatives immediately after the fish were caught. Therefore it is difficult to decide whether this failure to impregnate the nerves in the fish heart was due to delay in fixation giving rise to post mortem changes or was due to the nature of the nerve supply of the fish heart. However, from the sections which did show a few scattered nerves, it could be made out that in the fish nerves enter the ventricle along the bulbus arteriosus and along the wall of the A.V. funnel. Only a few nerves accompany the coronary arteries.

The ventricles of the vertebrate heart have been found to be profusely innervated except in the fish in which only a few nerves were observed. A comparative study of the sections of the various animals examined showed that the ventricles of the domestic fowl have the richest nerve supply (Fig.43). The photograph hardly gives an adequate impression of how profusely the avian ventricles are innervated as only a few nerves come into view at any given focus and the extremely fine branches cannot be recognised at the low magnification of the photograph.

It is claimed by Nonidez (1939) and supported by Naylor (1945) that with a modified Cajal technique, the sympathetic and parasympathetic fibres, especially in young animals could be differentially stained and their area of supply could be demarcated. By this method, sympathetic postganglionic fibres are stained light yellow to orange while their parasympathetic counter parts are stained deep brown to

Fig.42. Sagittal section of the frog heart showing the nerve bundles which reach the ventricle around the A.V. opening and A.V. funnel. (Bodian) X 60

Fig.43. Sagittal section of the left ventricular wall of the domestic fowl. Note the profusion of nerves in the section. Photograph hardly gives an adequate impression of the vast richness of nerves in the fowl ventricles.

(Gros-Schultze) X 140

black. And accordingly with this method Nonidez (1939, 1941, 1943) using young puppies, dogs and rhesus monkeys and Kaylor (1945) using guinea pigs, investigated the nerve supply of the heart and reached contradictory conclusions. The present writer, therefore, examined three rat hearts (adult, 37 days and 17 days old) after staining with Nonidez's (1939) modified Cajal silver method, to varify the findings of the above mentioned authors. He, however, found that both deeply stained so called parasympathetic and lightly stained so called sympathetic fibres were present in the ventricular wall . Both types of fibres were seen in the large bundles in the proximal part of the myocardial plexus of the ventricles (Fig.44) but the pale stained fibres could not be traced into the distal parts of the ventricles as they became finer and therefore could not be differentiated from the background which took the same light yellow colour. The dark stained so called parasympathetic fibres were observed in all parts of the ventricles. Fig.45 shows such deeply stained fibres in the left ventricular wall close to the apex of the heart. The inter--pretation given by Nonidez and Kaylor of the differences in the intensity of impregnation of the nerve fibres will be criticised later in the Discussion.

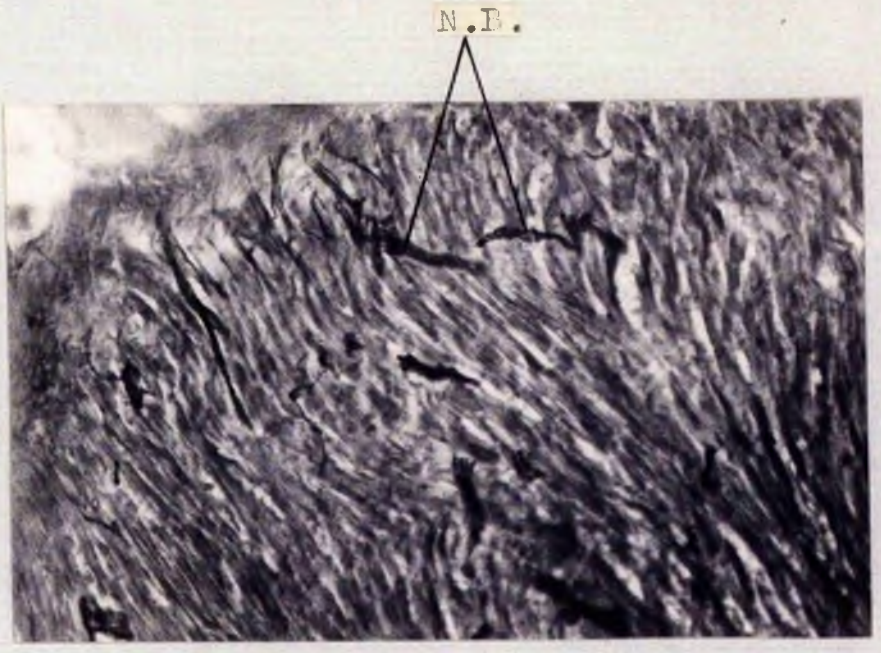

Fig.44. Oblique section through the rat heart showing bundles of nerves in the proximal part of the ventricular septum. These bundles contain both dark stained and light stained fibres.

(Nonides's modification of Cajal technique) X 230

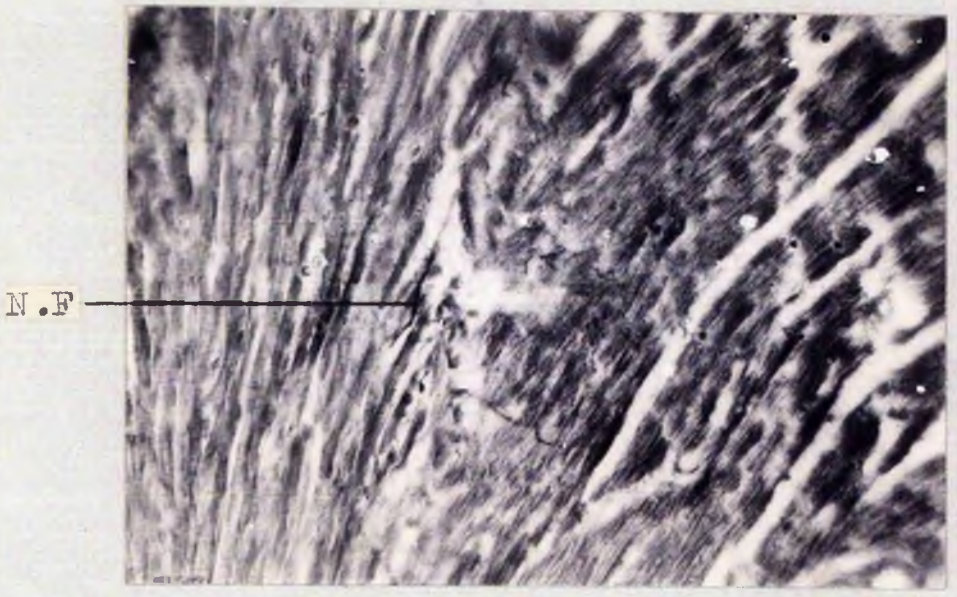

Fig.45. Oblique section through the left ventricular wall of the rat heart close to its apex. Segments of only dark stained nerve fibres can be seen in the centre of the picture.

(Nonides's modification of Cajal technique) X 230

INTRINSIC VENTRICULAR NERVE CELLS AND THEIR CONNECTIONS WITH THE PRE-GANGLIONIC FIBRES

Nerve cells, either singly or in the form of ganglia, are present in the ventricles of all the animals examined. They are comparatively more numerous in the dog, rat and domestic fowl than in the other animals in which there are only a few cells. The majority of these cells are situated in the basal portion of the ventricular wall adjacent to the A.V. sulcus. In the rest of the ventricular wall nerve cells are present but they are relatively scarce and are found mostly singly but occasionally in groups of two or three. Fig.47 shows a nerve cell in the left ventricular wall of the rat heart close to its apex.

In all animals these intrinsic ventricular nerve cells are generally situated subepicardially (Fig.46). In the dog, rat, domestic fowl and frog, they are also present in the myocardium of the ventricles (Figs. 48, 49, & 50) but such intramural nerve cells are only occasionally found. No intramural nerve cells were seen in the ventricle of the grass snake, lizard and fish. In the myocardium nerve cells, in most instances, are disposed either singly or in small groups of two or three.

Intrinsic nerve cells are frequently situated along the sides of large bundles of nerves, and fibres appear to pass from the cells to the nerve bundle and from the nerve bundle to intermingle with the cells of the ganglion (Fig.51), but these observations require confirmation by further evidence. Sometimes nerve cells are found in the course of

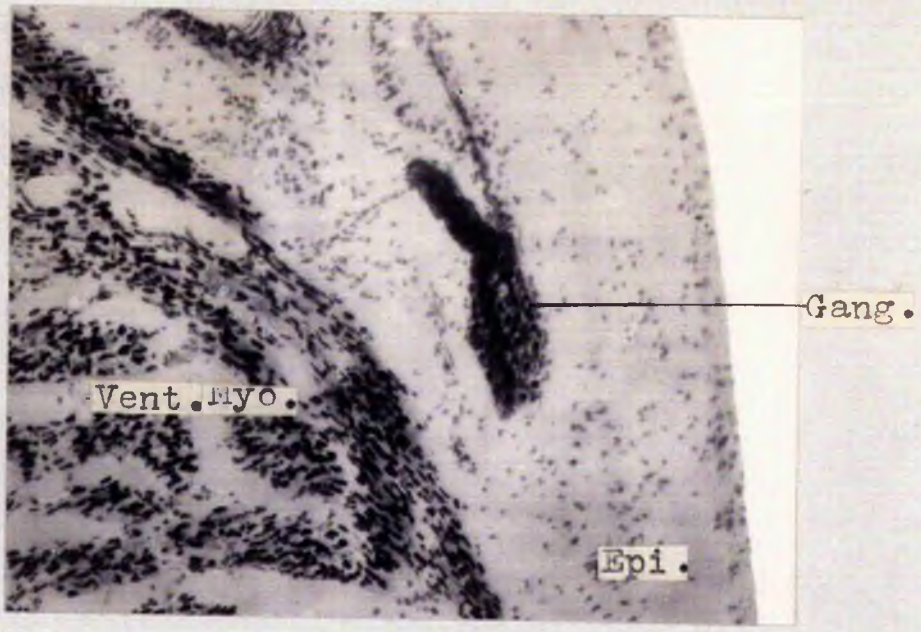

Fig.46. Coronal section through the left ventricular wall of the domestic fowl showing a group of ventricular subepicardial nerve cells close to the A.V. groove.

(Bodian) X 140

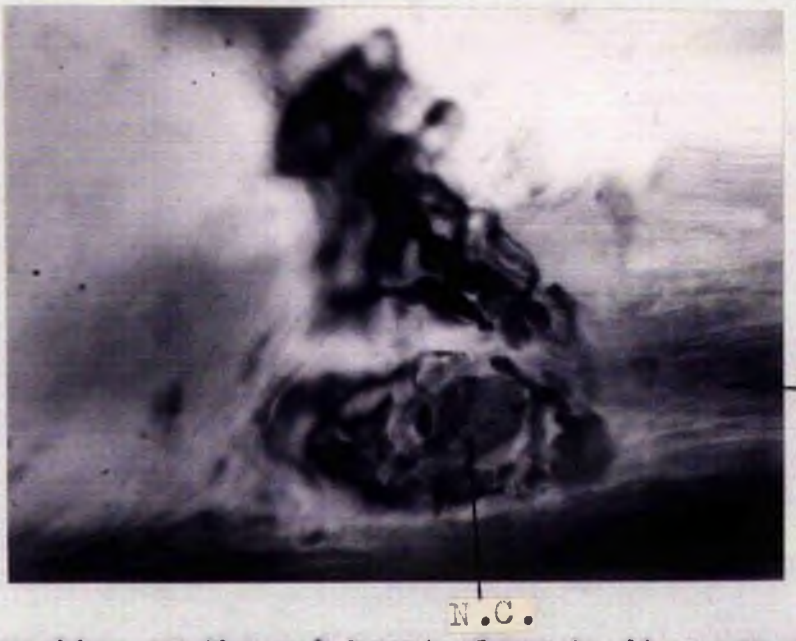

Fig.47. Transverse section of the rat heart close to its apex showing a nerve cell in the myocardium immediately close to the epicardium.

(Gros-Schultze) X 1100

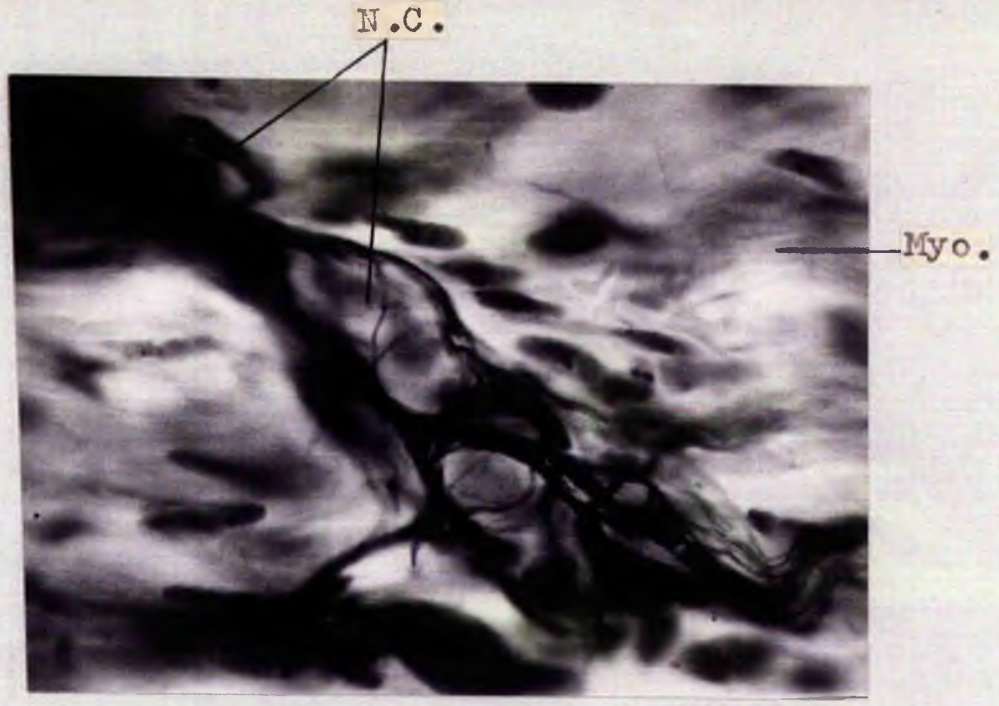

Fig.48. An oblique section of the rat heart showing intramural nerve cells in the ventricular septum. Cells are surrounded by the nerves of the myocardial plexus.

(Gros-Schultze) X 1100

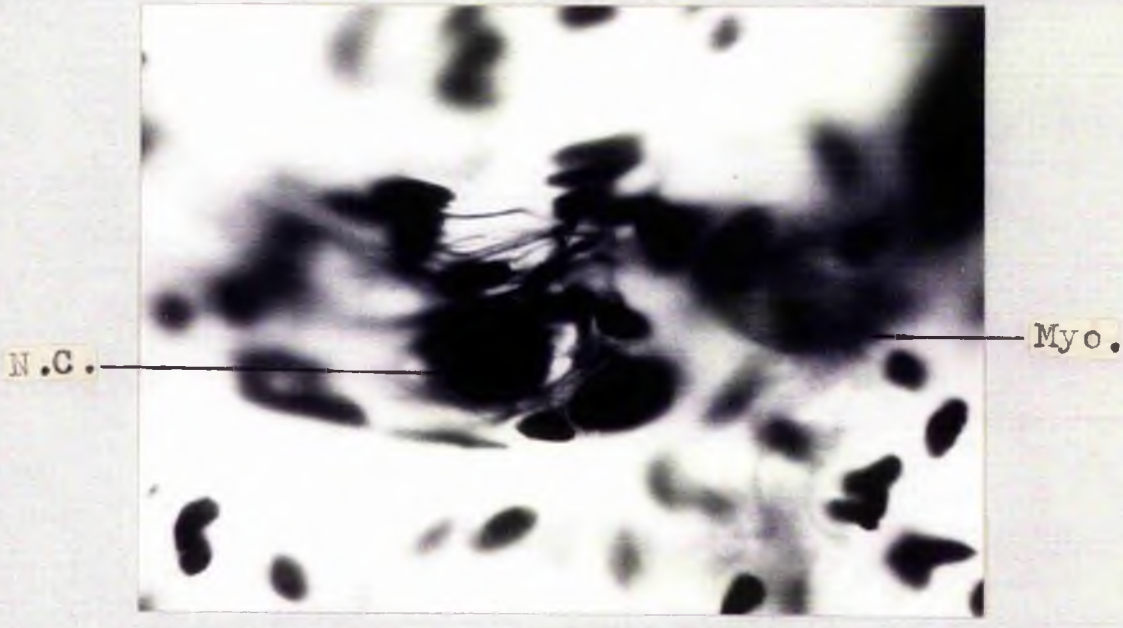

Fig.49. A sagittal section of the left ventricular wall of the domestic fowl showing two intramural ventricular nerve cells.

(Gros-Schultze) X 1100

a nerve bundle itself (Fig.52).

In most cases the exact character of these nerve cells could not be ascertained with any degree of certainty as their processes were frequently not clearly impregnated with silver and remained rather ill defined, although the cell bodies, nuclei and nucleoli were distinctly impregnated. However, it could still be decidedly recognised that the majority of the intrinsic ventricular nerve cells are multipolar in character. The cell bodies are irregularly polygonal or star-shaped (Fig.53) with round or oval nuclei and have a number of processes which vary from three to eight. The processes come out from all around the cell, thus giving the cell body a stellate appearance. Some nerve cells, however, showed an oval or pear-shaped cell body with round somewhat eccentric nucleus. A vague single process or a number of ill defined processes appeared to arise from the tapering pole of such cells (Fig.54). Some of these pear-shaped cells are truly multipolar with all the processes emerging from one pole of the cell. It could not be made out with certainty whether all such pear-shaped cells are multipolar or whether some are unipolar. Likewise a few nerve cells, especially those seen along the course of nerve bundles (Fig.52), gave the appearance of being bipolar but their true nature could also not be established with certainty.

The processes of the cells which appear to pass out of the ganglion into one of the related nerve bundles get mixed with the other fibres of the bundle and hence could not be followed for a long

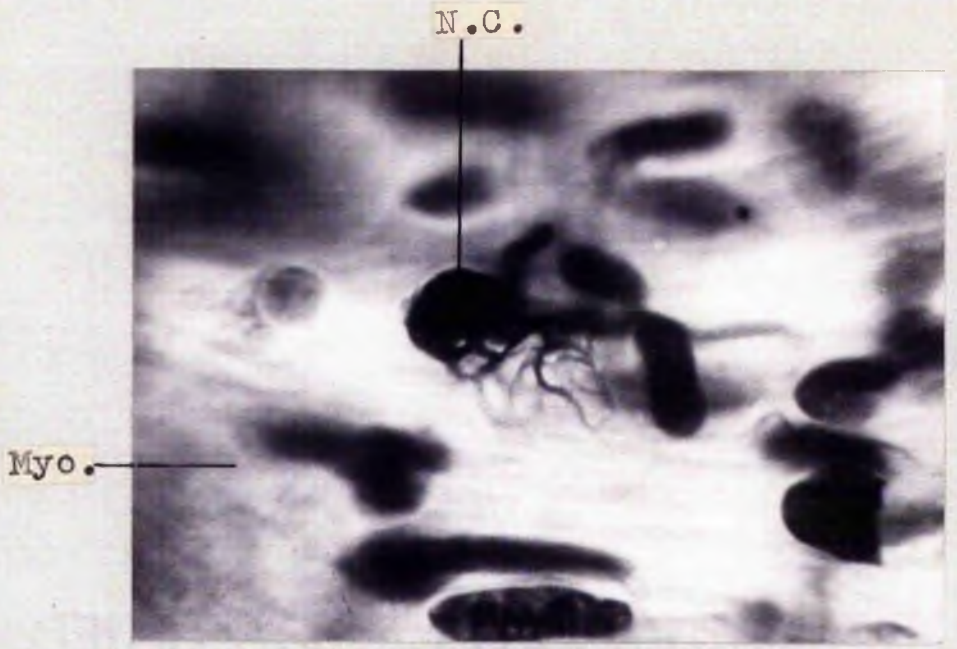

Fig.50. Sagittal section of the frog ventricle showing an intra-mural nerve cell in its proximal part.

(Gros-Schultze) X 1200

Fig.51. A group of subepicardial nerve cells over the left ventricular wall of the fowl's heart, close to the A.V. sulcus. Note the capsule of neurolemmal (satellite) cells around each nerve cell and the intricate arborisation of nerve fibres in the ganglion.

(Gros-Schultze) X 750

distance. The exact relationship between the axon of a ganglion cell and the structure innervated by it could not be ascertained as no process from any of the cells could be traced to its destination.

A large number of secondary cells resembling Schwann (satellite) cells and connective tissue cells form a sort of a capsule around each ganglion (Fig.51). These capsular cells are more numerous in the domestic fowl than in other animals. Secondary extension of this capsule penetrate between the individual nerve cells of the ganglion forming a sheath of satellite cells around each of them. The pre-ganglionic fibres coming to the ganglion form very intricate arborisations among these satellite cells and around the nerve cells of the ganglion. It is difficult to trace an individual nerve fibre to a particular nerve cell and to ascertain its precise relationship with it. However, as far as could be made out from the study of a number of sections, the preganglionic fibres appear to end in the ganglion in the following way. These fibres, many of which divide into several branches, pass tortuously among the satellite cells, over and between the nerve cells and ultimately terminate sometimes freely and sometimes with a slightly bulbus end in contact with the body of the nerve cell or its capsule of satellite cells (Fig.51). So far as could be ascertained these endings represent the synaptic connections between the preganglionic fibres and the ganglion cells.

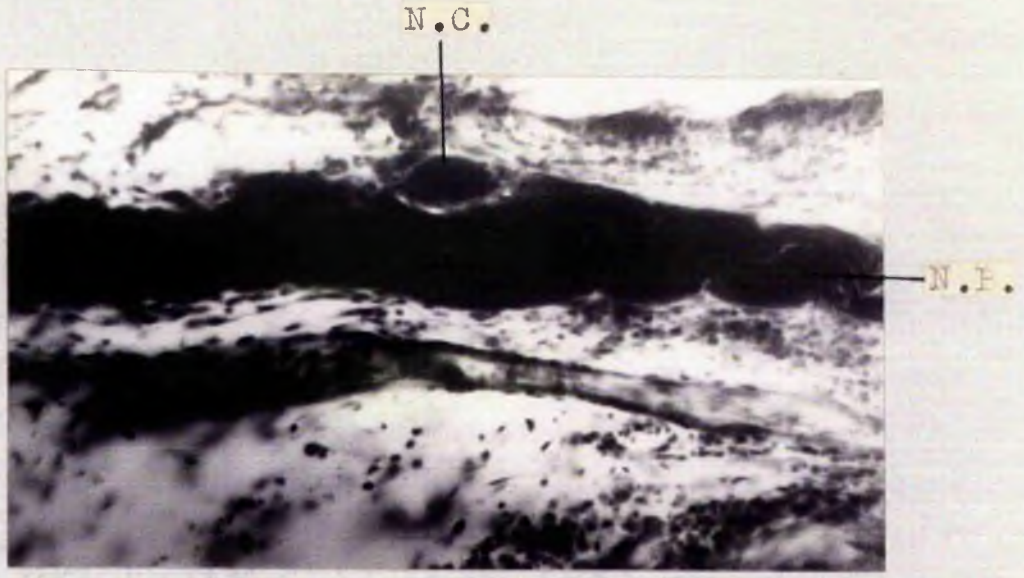

Fig.52. **A nerve cell in the course of a nerve trunk in the epicardium of the right ventricle of the domestic fowl.**

(Cros-Schultze) **X 220**

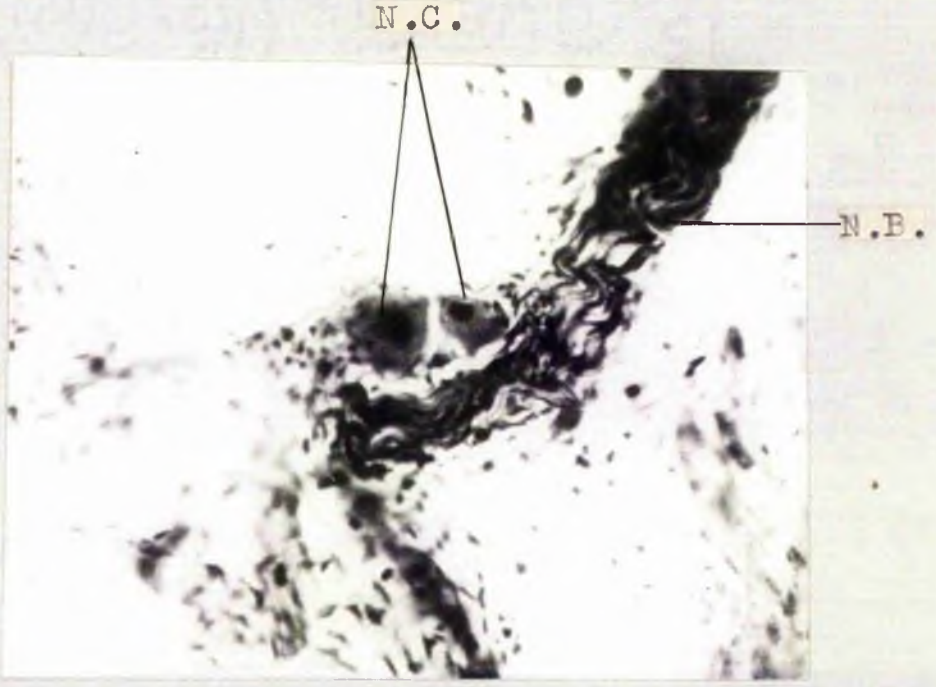

Fig.53. **Two multipolar ventricular nerve cells in the subepicardium of the left ventricle of rat. Note that the cells appear angular in shape** due to their **processes which are not very clearly impregnated with silver.**

(Cros-Schultze) **X 350**

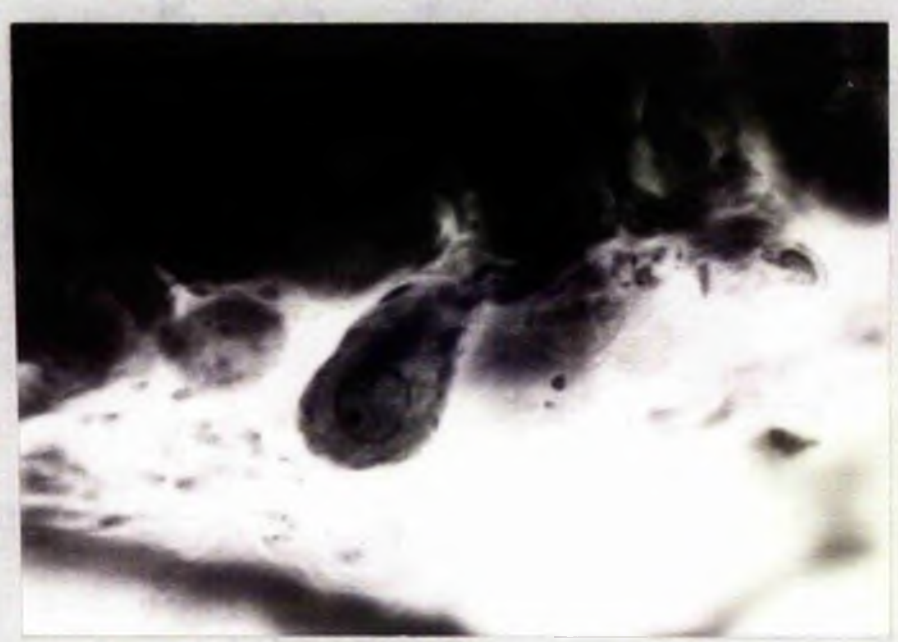

Fig.54. A subepicardial pear-shaped nerve cell in the A.V. groove of the rat heart. Darker cells on the upper part of the picture are the other cells of the ganglion. Indistinct processes appeared to have arisen from the tapering pole of the cell.

(Cros-Schultze) X 850

END-FORMATIONS OF THE CARDIAC NERVES IN THE VENTRICLES

Bundles of nerve fibres emerge from the subepicardial, myocardial and subendocardial plexuses. These continue to divide and subdivide into smaller bundles until finally the nerve fibres are running singly or in pairs. These fibres are connected with the strands of the terminal nerve network present in the various layers of the ventricular wall. Definite organised afferent or efferent endings resembling muscle spindles or motor end-plates as found in the skeletal muscles, were not found. Similarly no definite nerve endings in the form of end bulbs or loops, either in the cytoplasm or on the surface of the muscle fibres could be discovered in the ventricular wall. The cardiac nerves are related to the various components of the ventricles through strands of the fine terminal nerve network formed by very fine beaded fibres (Figs. 55, 56, 57 & 58). These constituent fibres of the network are much smaller in diameter than the fibres seen in the bundles of nerve fibres in which also the fibres do not show any anastomotic connections. One or two relatively more deeply impregnated fibres which had also a varicose appearance were observed to pass through these strands of the terminal nerve network. These deeply impregnated fibres resemble the fibres of the myocardial and other ventricular plexuses. Many nuclei are associated with the strands of network and there is some very faintly impregnated cytoplasm with indefinite boundaries around these nuclei. These nuclei are of different sizes and shapes. Some are round (Fig.57), some are elongated (Fig.56) while still others are transitional between

Fig.55. Strands of terminal nerve network in the right ventricular wall of the rat. Note that only a few nuclei are associated with these strands of network.

(Bodian) X 1000

Fig.56. Strands of terminal nerve network in the myocardium of the frog ventricle. Note the elongated appearance of the nucleus seen in the upper strand.

(Gros-Schultze) X 1000

these two varieties. A few of them appeared to be somewhat triangular. The majority of these nuclei appear to be imbedded in the meshes of the network, with the constituent fibres of the network lying around them. In such a strand of the network the fine beaded fibres surrounding the nucleus give the appearance of a neurofibrillar cytoplasmic differentiation. In other places, however, the nuclei are situated at the margin of the network. Many of these nuclei, especially the elongated ones, are probably Schwann nuclei as they have the same appearance as those Schwann nuclei seen along the bigger bundles. The more rounded and the triangular nuclei are, however, considered by many as belonging to a special type of cells - known as the "interstitial cells".

The general pattern of the terminal nerve network appears to be the same in the ventricles of all the animals investigated, although in the individual animal it shows a certain amount of variation between the different parts of the ventricles. In some parts of the ventricular wall the strands of the terminal nerve network consist of faintly impregnated very fine beaded fibres, running close together in a wavy manner and almost parallel to one another with only little anastomosis between them (Fig.58). In other places very fine beaded fibres form strands in which the fibres frequently anastomose with one another and thus give the typical net-like appearance with associated nuclei lying in the meshes of the network (Fig.57). In some instances strands of network are relatively free from nuclei for a considerable length while in other places the nuclei are closer together.

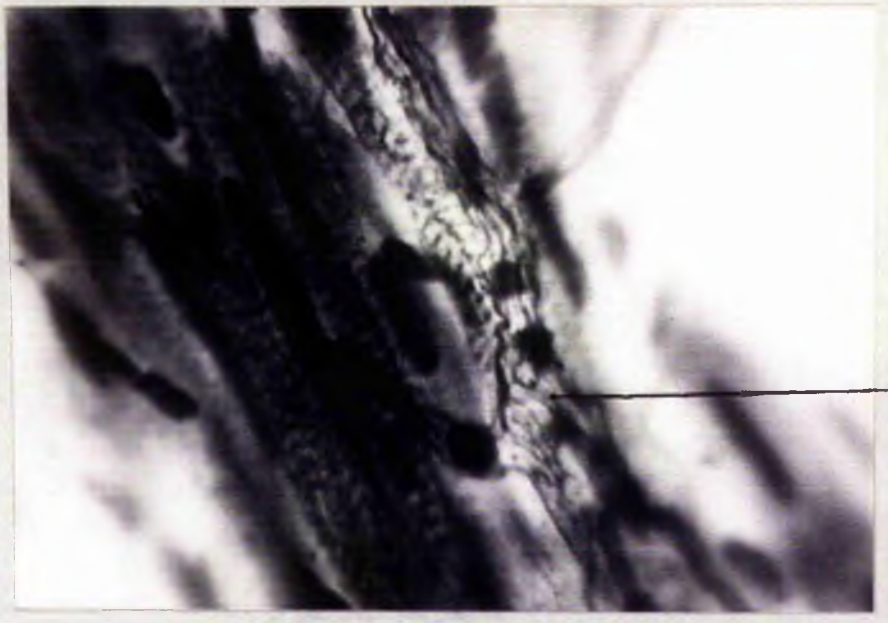

Fig.57. Strands of terminal nerve network in the left ventricular wall of the fowl heart. Note that the associated nuclei are round in shape and appear to be imbedded in the network.

(Bodian) X 1100

Fig.58. A strand of the terminal nerve network in the left ventricular wall of the fowl heart. Note the wavy character of the constituent fibres which run almost parallel to one another with only a few anastomoses between them.

(Gros-Schultze) X 980

Strands of the terminal nerve network traverse the ventricular wall in a zig-zag manner and with no particular relation to the axes of the myocardial fibres. As a result of their irregular course only small segments of the terminal network may be seen in any particular optical plane. When one examines a number of sections, one becomes convinced that these strands of the terminal nerve network pass in various directions in the ventricular wall and join one another to form a diffuse tri-dimensional network which pervades the various tissues of the ventricles and comes into contact with the effector cells.

It was occasionally observed especially in Gros-Schultze preparations that extremely thin fibres given off from the terminal nerve network which appeared to enter the muscle fibres. The writer is, however, not certain of this observation.

In the subendocardium and in the valves of the ventricles, strands of the same terminal network are present (Fig.59), and the closest scrutiny of my sections revealed no organised nerve endings in these parts of the ventricles.

Apart from these strands of the terminal nerve network described above, single thick medullated nerve fibres of a terminal character, are also present in the ventricles. These are seen in the myocardium sometimes running parallel to the muscle fibres and sometimes transversely to them. They are also present in the subendocardial connective tissue. These single fibres could be followed for a considerable distance by adjusting the focus of the microscope. They are generally deeply impregnated and could not be traced to any definite

ending. Instead, after a variable course they underwent a gradual thinning and ultimately disappeared from view (Fig.60). Some of these thick single fibres divide into two or three branches each of which, likewise becomes thinner and thinner and ultimately disappear. These thick medullated fibres are presumably sensory in nature.

The bulbus cordis which as a separate chamber is present only in the frog, has the same type of innervation as the ventricle.

Although no discrete and definite organised nerve endings could be found arising from any nerve fibre, small enlargements were occasionally observed at what appeared to be the ends of the nerve fibres but on adjustment of the focus or on following the nerve fibres through subsequent sections, these apparent endings were found to be either bends or cut ends of the nerve fibres.

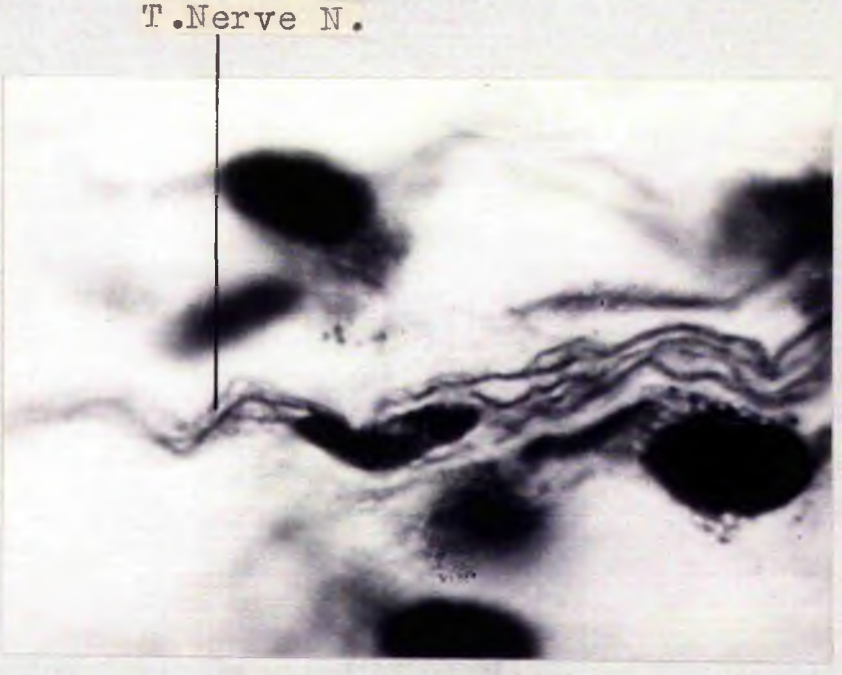

Fig.59. A strand of the terminal nerve network in the subendocardial tissue of the ventricular septum of the dog. Note the elongated shape of the nucleus which appears to be imbedded in the network.

(Gros-Schultze) X 1500

Fig.60. A single thick medullated nerve fibre in the ventricular septum of the dog. Note the wavy course of the fibre and its faint colour in the right half.

(Gros-Schultze) X 840

NERVOUS COMPONENT OF THE ATRIO-VENTRICULAR BUNDLE

Of the various animals studied in the present investigation the atrio-ventricular bundle is present only in the dog, rat and domestic fowl. Even in them the specific muscle tissue, especially in the proximal part of the bundle is histologically rather poorly defined. The muscle fibres of the A.V. bundle appear almost like those of the general myocardium except that they are impregnated more lightly in silver preparations. In the peripheral part where typical Purkinje fibres are present, ramifications of the bundle can be identified more easily. In the domestic fowl the Purkinje cells are situated in the myocardium as well as subendocardially but only subendocardially in the dog. In the ventricles of the rat heart typical Purkinje fibres could not be seen. The specialised conducting system is altogether absent in the lower vertebrates like lizards, frogs and fish, in which the atrial musculature is continuous with that of the ventricle through the A.V. funnel (mentioned in Part I), the muscle fibres of which are histologically similar to the rest of the myocardium.

In the dog, rat and domestic fowl, the A.V. bundle is accompanied by nerves, both in the form of bundles of nerve fibres and of a fine plexus. A comparative study shows that the A.V. bundle in the rat has the richest innervation (Fig.39) and the A.V. bundle of the dog has the least rich innervation (Fig.61) among these three species of animals showing the A.V. bundle. The nerves and the muscle fibres of the bundle are so thoroughly intermingled with one another,

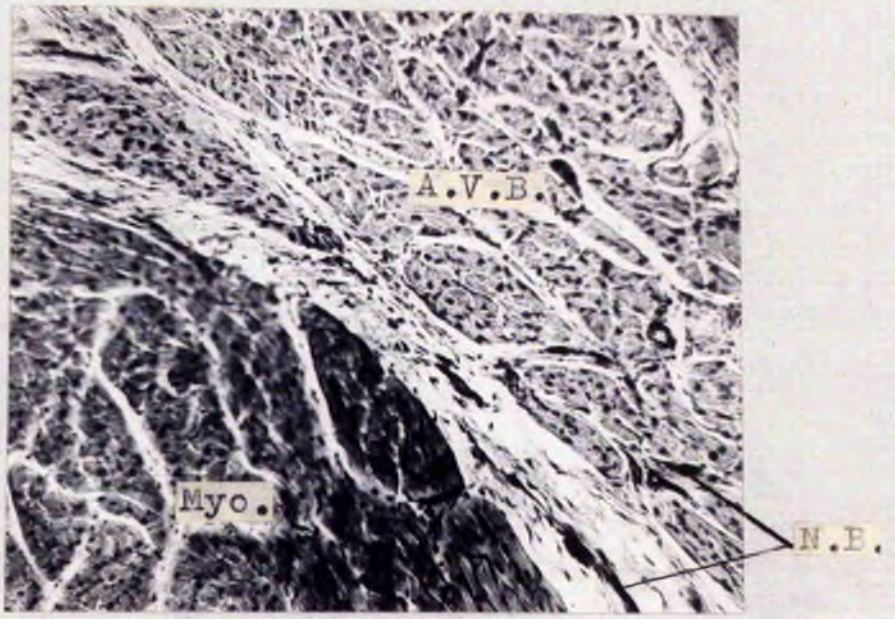

Fig.61. Sagittal section through the proximal part of the ventricular septum of the dog, showing the main body of the A.V. bundle. Note the paucity of nerves in the bundle and also the differentiation of its muscle fibres from those of the septal myocardium.

(Gros-Schultze) X 120

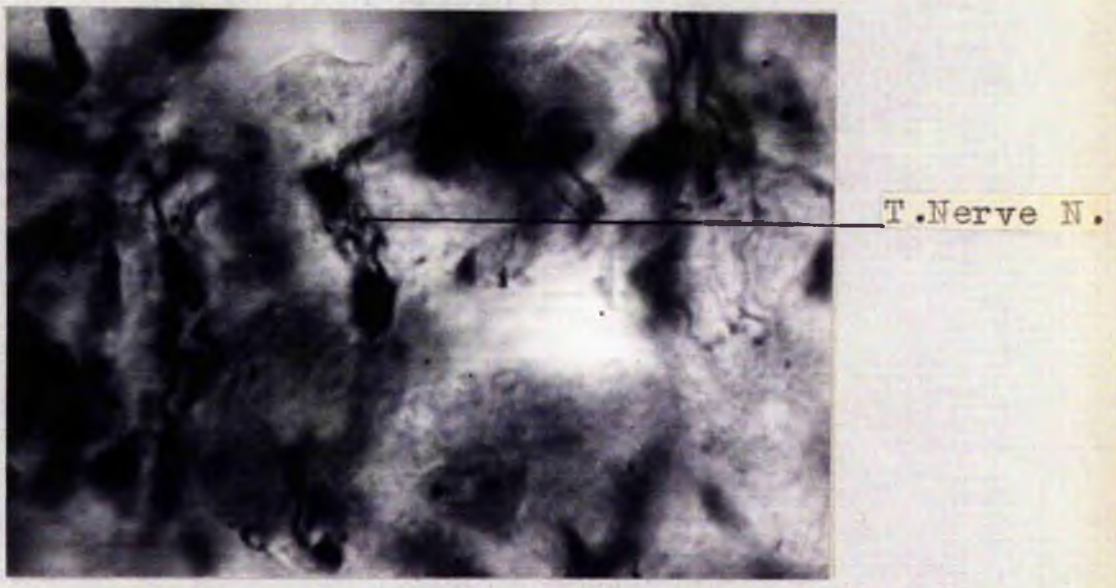

Fig.62. A high power view of the enclosed area in Fig.39 showing strands of terminal nerve network in the proximal part of the A.V. bundle of the rat heart.

(Gros-Schultze). X 1100

especially in the rat, that the two components together could be termed a neuromuscular bundle.

The peripheral Purkinje fibres appear to be without any nerve supply, because although both bundles of nerve fibres and strands of the terminal nerve network were sometimes observed quite close to these terminal Purkinje fibres, they were never seen actually in contact with them in any of my preparations.

No organised nerve endings of any size or shape are present in the A.V. bundle. Strands of fine terminal nerve network with imbedded nuclei, similar to those found in the other parts of the ventricular wall , are also present in the A.V. bundle (Fig.62).

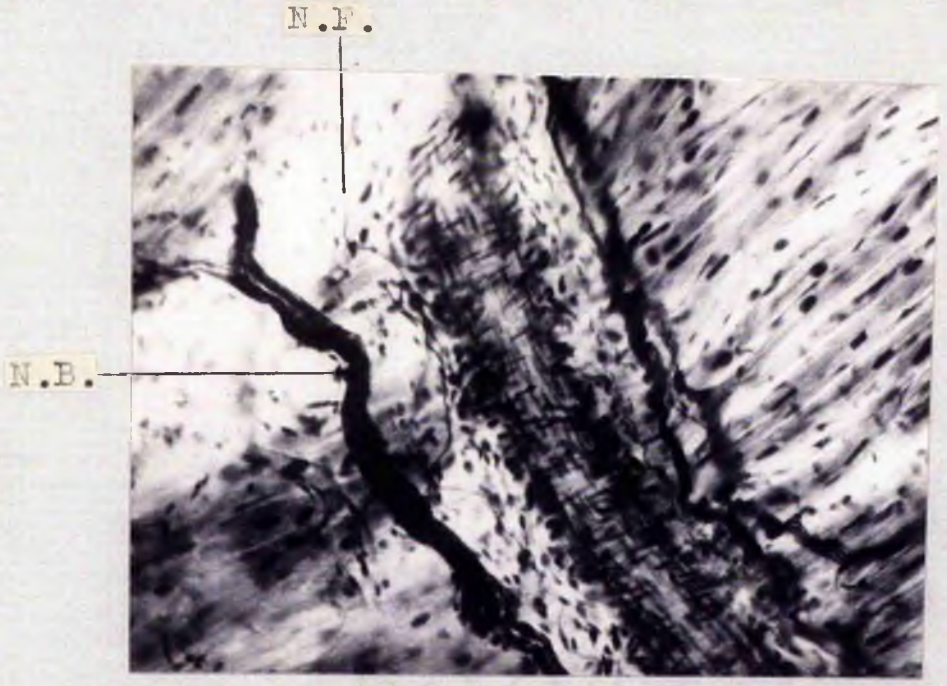

Fig.63. An oblique section of the left ventricular wall of the rat heart showing a branch of the coronary artery with its perivascular nerve bundles. Note that the branches from this perivascular plexus pass to the vessel wall and supply it.

(Gros-Schultze) X 230

Fig.64. Transverse section of a branch of the coronary artery in the dog's left ventricular wall. Note that the intima and the deeper part of the media are devoid of any nerve.

(Gros-Schultze) X 60

INNERVATION OF THE CORONARY ARTERIES

In the dog, rat and domestic fowl, coronary arteries and their branches are surrounded by very rich plexuses of nerves, which are known to be formed by both sympathetic and parasympathetic nerves. In reptiles (grass snakes and lizard), amphibia (frog) and fish (cod and haddock), in which the coronary arteries are relatively poorly developed, the coronary plexuses are also rather poor. The nerve bundlesof these plexuses entwine and surround the coronary arteries in an intricate manner. These bundles of nerve fibres consist of both thick medullated fibres and fine apparently non-medullated fibres. Some are of uniform thickness and others show varicosities. These perivascular nerves run mostly in the connective tissue surrounding the arteries and also in their adventitial coat. Fig.63 shows bundles of nerves accompanying a branch of the coronary artery in its surrounding connective tissue. Smaller branches from the nerve bundles approach the vessel wall to supply it. These perivascular nerves, as had already been mentioned, not only supply the blood vessels but they are also the chief source of the nerves of the myocardial plexus. As in the rest of the ventricle, there are no organised nerve endings present in the vessel wall . Instead, fine strands of the terminal nerve network with imbedded cell nuclei were observed in the adventitia and the immediately adjacent part of the media (Fig.65). In addition to the strands of the terminal nerve network, single thick medullated nerve fibres without any definite endings are also present (Fig.66). Sometimes in occasional

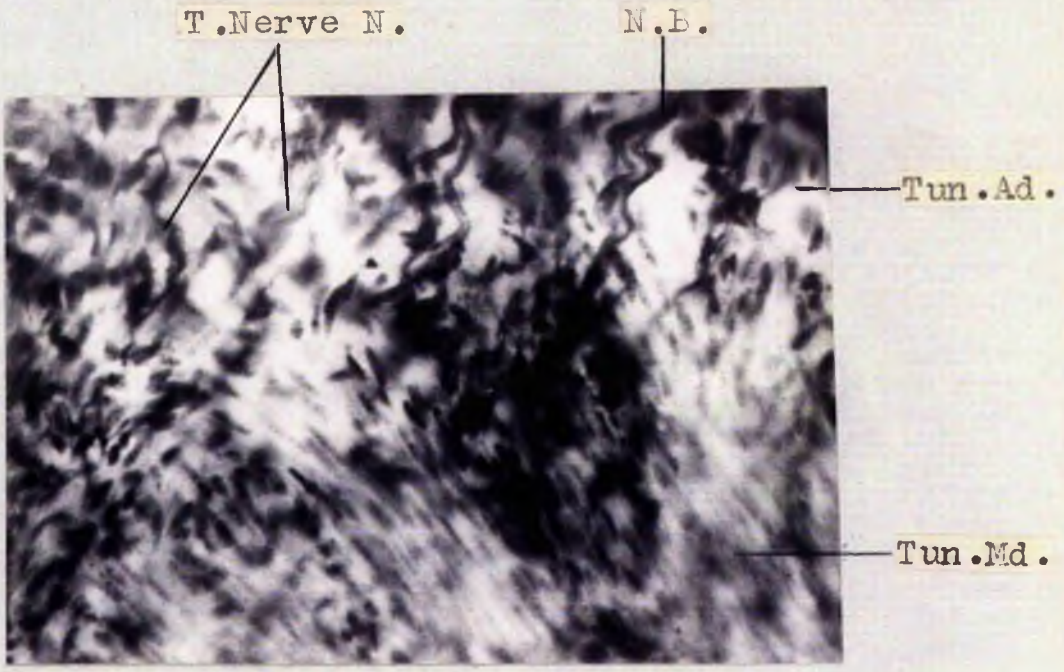

Fig.65. Higher magnification of a portion of the vessel shown in Fig.64. Note that the nerve supply of the vessel is confined to the adventitia and the subjacent part of the media (upper half of the photograph).

(Gros-Schultze) X 350

Fig.66. Thick single nerve fibres in the adventitia of a branch of the coronary artery. Deeper portion of the media (left portion of the picture) shows no nerve elements.

(Gros-Schultze) X 900

places cut ends of these thick nerves gave the appearance of a flattened ending but adjustment of focus showed the true nature of such apparent endings. These single thick fibres become gradually thinner and more faintly impregnated with silver, and ultimately disappear.

The nerve supply of the coronary arteries appears to be limited to the adventitia and the immediately adjacent part of the media as no nervous elements were seen in the depth of the media nor in the intima (Figs.64 & 65).

In the myocardium of the dog, rat and domestic fowl, there is a rich network of capillaries. These capillaries of the myocardium run mostly in the direction of the muscle fibres and are connected with one another by transverse branches. The capillaries are so profuse that muscle fibres and capillaries almost alternate with one another. In close relation to the capillaries there are strands of the same terminal nerve network which supplies the other components of the ventricles. Figs. 67 and 68 show such strands in contact with capillaries. This nerve network surrounds the capillaries but never, as far as could be seen, gives rise to a nerve ending on the capillary wall.

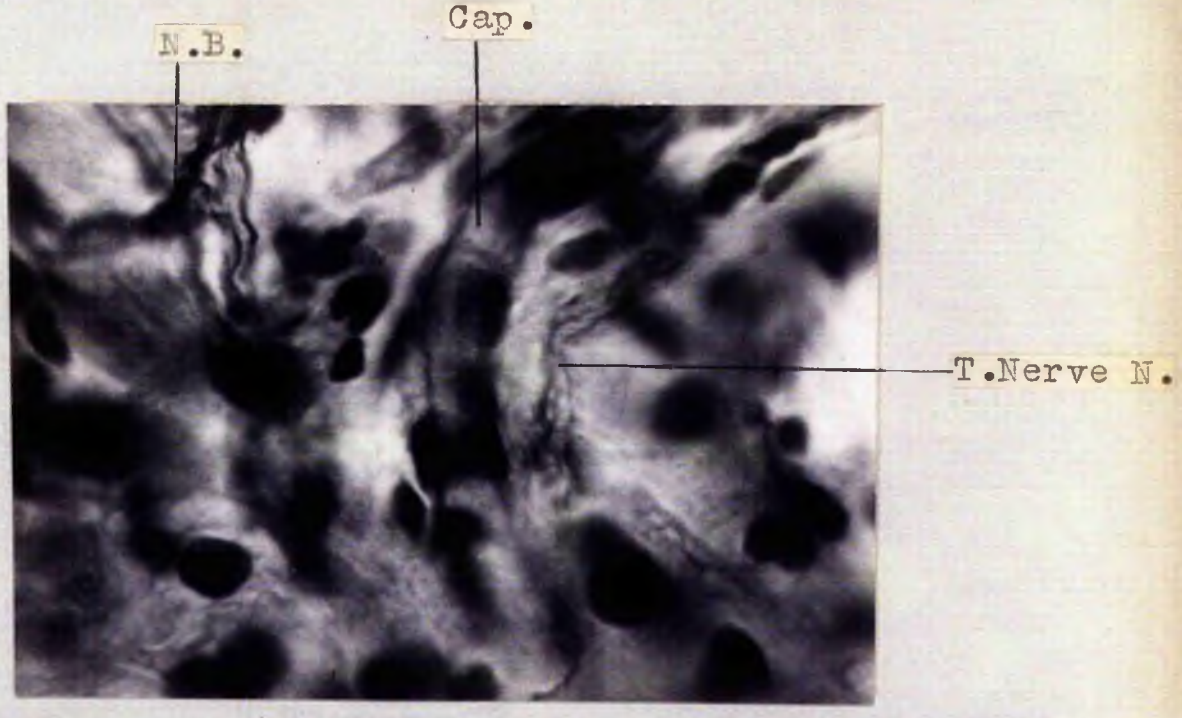

Fig.67. Strands of terminal nerve network with associated nuclei are seen in relation to a capillary in the left ventricular wall of the rat.

(Gros-Schultze) X 1200

Fig.68. Strands of terminal nerve network, surrounding a capillary in the proximal part of the left ventricular wall of the rat.

(Gros-Schultze) X 1200

DISCUSSION

DISCUSSION

Although the basic anatomical scheme of innervation of the cardiac ventricles in all vertebrates is similar, the mode of entrance of the nerves to the ventricle in the lower vertebrates appears to differ slightly from that in the higher. In the lower vertebrates (reptiles, amphibia and fish), most of the nerves enter the ventricle independently of the coronary arteries, whereas in the higher vertebrates (mammals and birds) the majority of the nerves have been found to reach the ventricles through the plexuses surrounding the coronary arteries and their branches as noted by previous workers for mammals (Lee, 1849; Woollard, 1926; Stöhr, 1932; Davies et al., 1952, and Mitchell, 1956). This difference is probably due to the fact that the coronary arteries in the lower forms are comparatively rudimentary and are for the most part limited to the relatively compact cortical portion of the ventricular wall, the deeper portion being spongy. In the frog the coronary arteries are completely confined to the bulbus cordis. As these arteries become more developed and branched in association with the evolution of the ventricular wall which becomes more compact in the higher forms, they are more widely utilised by the nerves as highways to the various parts of the ventricles.

Most investigators of cardiac innervation have noticed as the writer did, that large bundles of nerve fibres are arranged in a general plexiform manner in the epicardium, myocardium and endocardium before their ultimate distribution to the various parts of the ventricles (Woollard, 1926; Stöhr, 1932; Toheng, 1951, and Mitchell, 1956).

Rich innervation of the ventricles as observed in the present investigation, has also been recorded by other workers (Woollard, 1926; Boeke, 1932 and Stöhr, 1932). In the literature, however, there appears to be little recognition of the relatively very rich nerve supply of the avian ventricles. In the domestic fowl they are so richly innervated that every low power microscopic field of a section through the ventricular wall was literally studded with nerves. It is difficult to assign any reason for this vast richness of the ventricular nerves in the bird . The writer, however, is of the opinion that since the avian heart, especially that of the flying bird, is the most rapid beating of all vertebrate hearts, it probably requires a very sensitive regulation of its function, and hence the richness of its innervation.

The idea held by many workers that the ventricles are supplied only by the sympathetic or by the parasympathetic and not by both, has been debated by others. Woollard (1926) and Nonidez (1939, 1941, 1943) found that the ventricles were supplied predominantly by the sympathetic and received no or very few, if any, parasympathetic fibres. Kaylor (1945) and Lavrentiev (1946) found only parasympathetic vagal fibres in the ventricles. Using Nonidez's (1939) technique and his interpretation of the result obtained by it, the writer has found in three rat hearts that both sympathetic and parasympathetic fibres supply the ventricles. This agrees with the observations of Blackhall-Morison (1926) and Tcheng (1951) who also found both excitatory and inhibitory nerves in the ventricles.

The writer agrees with Nonidez (1939) and Kaylor (1945) that some of the nerves of the heart are impregnated faintly and some deeply by the Nonidez technique. These fibres have been interpreted by Nonidez as sympathetic and parasympathetic respectively. The writer has noted, however, that occasionally the same fibre may stain faintly in one place and deeply in another, and that only the thicker nerve fibres are impregnated by this technique. These points taken in association with the discrepancies in the findings obtained by other workers using this technique indicate that the results obtained by this technique are to be considered unreliable.

As regards the topography of the intrinsic cardiac ganglion cells, it is generally believed that in the mammal, the nerve cells are confined to the atrial epicardium (Meiklejohn, 1914; Woollard, 1926; Stohr, 1932, 1957; Seto, 1936 and Sato, 1954). However, Woollard did find ventricular epicardial nerve cells in the grass snake. Davies et al (1952) found ventricular epicardial nerve cells only in Artiodactyle and Cetacea among the many orders of mammals studied by them. There are others who describe a more widespread distribution of the cardiac nerve cells, extending to the ventricles also (Berkley, 1894; Mollard, 1908; Wilson, 1909; Jones, 1927; King, 1939; Toheng, 1951 and Mitchell et al, 1953). The writer likewise has found intrinsic nerve cells in the ventricles of all the animals he has examined except in the ventricle of fish. As the writer has not had much success in staining the nervous elements in the fish ventricle for the reasons already stated, he puts no reliance on the present

negative finding in the fish

Woollard (1926) and Davies et al. (1952) contended that all cardiac nerve cells are situated only subepicardially and denied the presence of intramural nerve cells. The candidate, however, agrees with King (1939), Tcheng (1951) and Mitchell (1956) that intramural nerve cells, though relatively few, are definitely present in the ventricles of the higher vertebrates. Thus from a review of the literature and from the present findings I feel it is safe to conclude that intrinsic nerve cells, though few in number, are present in the cardiac ventricles. They are situated mostly subepicardially, particularly close to the A.V. sulcus and are also present intramurally in the ventricles of birds and mammals (Figs. 48 and 49).

The majority of the ventricular ganglion cells found in the present investigation, appeared to be multipolar in character, although four or five cells were observed which were possibly unipolar or bipolar in form. The presence of unipolar or bipolar nerve cells could not, however, be established with certainty. Woollard (1926) noted that the cardiac nerve cells are principally multipolar and only occasionally unipolar. Berkley (1894) observed some bipolar cells in the cardiac ganglia. Davies et al., (1952) found that the epicardial nerve cells are mostly bipolar or unipolar in type, only a few being multipolar whereas those related to the A.V. bundle are principally multipolar, only a few being bipolar or unipolar. Mitchell et al.,(1953) concluded that ventricular epicardial nerve cells are predominantly multipolar. It should be noted in this connection that often it is difficult to decide to what type a nerve

cell belongs, since their processes often do not lie in the same optical plane as the section and also are frequently not very clearly impregnated with silver. Furthermore it has not been possible to trace the processes of an individual cell to their destination. I think these points may account for the variations in the findings of different investigators.

Various types of sensory endings have been described in the heart by different investigators. Woollard (1926) saw free and brush-like endings in the epicardium and endocardium. He also found the long single dendrite of some of the intrinsic nerve cells terminating as a complicated nerve net which he interpretated also as a type of sensory ending. Stöhr (1932, 1957) described encapsulated and arborising endings in the epicardium. Muscle spindles and encapsulated sensory endings in the rat ventricles were described by King (1939). Nonides (1941) found minute bulb and loop-like endings. Davies et al.,(1952) have noted only simple knob-like sensory endings in the epicardium and endocardium. No such organised sensory endings were seen by the writer in any of his preparations. He has, however, observed single thick apparently medullated fibres presumably sensory, which run very tortuously and gradually become thinner and finally disappear from view. They are present in all the layers of the ventricles. Sometimes such a fibre divides into two or three branches each of which similarly becomes thinner and gradually disappears. Seto (1936), Davies et al.,(1952) and Sato (1954) have also recorded similar findings. In addition to these single medullated fibres

strands of the terminal nerve network, to be discussed presently, were also observed in all the layers of the ventricular wall including the subendocardial tissue and the valves. A similar distribution of the nerve network is also described by other workers (Stöhr, 1932, 1957; Landau, 1950; Meyling, 1948, 1953; Mitchell, 1953; Sato, 1954 and Holmes, 1956, 1957). Recently Mulligan (1957), using Gros-Schultze technique, has observed cords of nervous terminal reticulum with associated nuclei in the ligaments attached to the vertebral bodies of the dog. He believes these cords of reticulum to be the end-formations of the autonomic nerves in these ligaments. Apart from these cords of terminal reticulum he has also found nerve fibres which end by tapering or by unravelling of fibrils and these he considers as probably receptor in character. These findings indicate that the terminal nerve network is partly sensory in character.

As regards the termination of the myocardial nerves, I could find no organised ending lying either on the surface of the muscle cells or inside their protoplasm, similar to those observed by some of the previous workers. (Woollard, 1926; Jones, 1927; Nonides, 1939, 1941, 1943; King, 1939; Stotler and McMahon, 1947; Tcheng, 1951 and Davies et al., 1952). Apart from the single medullated fibres just discussed, the only other terminal nervous structure I did find consisted of strands of the terminal nerve network. These strands consist of a network of very fine nerve fibres associated with nuclei surrounded by scanty faintly impregnated cytoplasm. In some places one or two relatively more deeply impregnated beaded

fibres were seen running through the fibrillar nerve net comprising these strands. These deeply stained fibres are considered by Meyling (1953) to be the terminal ramifications of the extrinsic cardiac nerves and the postganglionic fibres of the intrinsic cardiac ganglion cells making connections with the terminal nerve network. These strands of the terminal nerve net are present in all the layers of the ventricular wall.

The idea of a terminal nerve network is not a new one and many other previous workers have observed it in the cardiac ventricles and also in the other vegetatively innervated viscera. They similarly regard it as the ultimate end-formation of the autonomic nerves in a viscus (Stöhr, 1934, 1935, 1957; Boeke, 1932, 1949; Seto, 1936; Li 1940; Akkeringa, 1949; Field, 1951; Mitchell, 1953, 1956; Meyling, 1938, 1948, 1953; Sato, 1954; Kuntz and Napolitano, 1956; Honjin, 1956, and Holmes, 1956, 1957; and Mulligan, 1957). The terminal nerve network observed in the present investigation is morphologically the same as the "pre-terminal and terminal reticulum" of Stöhr and Reiser, the "sympathetic ground plexus" of Boeke and the "neural net" of Honjin.

Nonidez (1936, 1937 and 1939), on the other hand considers that this terminal network is not neural in character but is formed by argyrophilic reticular tissue. Boeke (1938, 1949) and Meyling (1953) have answered this criticism and stated that the argyrophil reticular fibres have quite a different appearance to that of the terminal nerve network. The writer agrees with this view of Boeke and Meyling as

he has also seen, in some of his discarded Gros-Schultze preparations in which the connective tissue fibres were stained, that the connective tissue fibres had a peculiar appearance of their own and were irregularly scattered all over the sections and were not arranged in strands as are the fibrils of the terminal nerve network.

Weddell and Zander (1950, 1951) believe that the terminal nerve network is an artefact due to formalin fixation. Mitchell (1953) has, however, shown that this view is incorrect as he could stain the nerve network and its associated cells with intravital and supravital methylene blue, in tissues which have not been treated with formalin at any stage of their preparation.

The individual organised nerve endings seen by some of the previous workers are regarded by others, who believe in the presence of a terminal nerve network, as artefacts due to imperfect impregnation of the nerve network.

The mode of formation of the terminal nerve network and the character of the cells associated with it are likewise a matter of controversy. Stöhr (1935, 1957), Seto (1936), Sato (1954), and Kuntz and Napolitano (1956) believe that the network is formed by the terminal ramifications of the sympathetic and parasympathetic post: ;ganglionic fibres and is associated with interstitial cells and Schwann cells. Hillarp (1946) regards it as composed of Schwann plasmodium with individual sympathetic and parasympathetic post: ;ganglionic fibres running through the plasmodial network. Li (1940), Akkeringa (1949), Meyling (1953) and Honjin (1956) regard that the

terminal nerve network is composed of the interlacing processes of nerve cells, called interstitial cells and that the ortho and parasympathetic post-ganglionic fibres make synaptic connections with it.

Regarding the cells which are present in association with the terminal nerve network, Stöhr (1935, 1957), Boeke (1949), Mitchell (1956) and others think that both interstitial cells and Schwann cells are present in the terminal nerve network. Stöhr and Mitchell regard as interstitial cells only those which are situated at the intersections of the strands of the network and the rest are regarded by them as Schwann cells. Akkeringa (1949), Meyling (1953) and Honjin (1956) however, are of the opinion that all cellular elements of the terminal nerve network are interstitial cells. Leeuwe (1937) and Champy, et al., (1945-46) after a detailed study with various techniques also arrived at the same conclusion namely that the cells are all neural in character and not neurilemmal sheath cells or connective tissue cells.

Some workers (Woollard, 1926, and Nonidez, 1936, 1937, 1939, 1944), it may be pointed out who deny the very existence of a terminal nerve network and regard such a network as composed of argyrophil connective tissue fibres, believe that the cells present in association with the network are connective tissue cells.

It is rather difficult to give a definite answer to these controversial questions from the findings of the present investigation in which only silver impregnation techniques were used and no experimental

work was done.

The specialised conducting tissue of A.V. bundle is absent in the ventricle of the lower vertebrates. The writer could not find muscle fibres histologically different from ordinary cardiac muscle fibres in the ventricles of the grass snake, green lizard, frog and the fish, such as are found in the dog, domestic fowl and rat. This confirms the observations of Davies and Francis (1941 and 1946) and Davies (1942) who likewise found that the specialised conducting tissue is present only in the higher vertebrates viz. birds and mammals. On the basis of this finding, Davies postulated that the cardiac conducting system has evolved simultaneously with the more rapid rate of the heart in these homoiothermal animals. Davies, et al.,(1952) could not also find any specialised conducting system in the crocodilian heart.

The nervous elements of the A.V. bundle of the animals in which the conducting system is present, differ from species to species. In the present investigation it has been found that the rat's A.V. bundle is profusely innervated whereas that of the dog has only a few nerves in it (Figs. 39 & 61). Meiklejohn (1913) and Field (1951) have described a similar species difference in the nervous component of the conducting system. Blair and Davies (1935) found a rich innervation of the conducting system of the ox heart but a relatively poor innervation in man. Toheng (1951) found only a few nerve fibres in the main A.V. bundle of the dog and the branches of the bundle were devoid of nerves. Davies, et al., (1952) while studying the

ventricles of many mammalian orders observed numerous nerves accompanying the A.V. bundle in Artiodactyla and Cetacea, but only a few nerves with the A.V. bundle in the other mammals investigated by them.

The appearance presented by the terminations of nerve fibres in the A.V. bundle, as elsewhere in the ventricular wall, has been variously interpretated but no interpretation has been generally accepted. Stotler and McMahon (1947) have observed discrete flattened and complicated nerve endings in the proximal part of human A.V.bundle . Davies et al.,(1952) found two types of endings in relation to the muscle fibres of the A.V. bundle . The first type was a fine plexus of nerve fibres, applied on the surface of the muscle fibres. They consider this fine plexus as sensory in nature. The second type was a simple knob-like ending, which was seen on the surface of only some of the bundle muscle fibres. They suggested that this second type of ending might be motor in nature. Nonidez (1943) described ring-like and reticulated endings in the A.V. bundle of the monkey. In the present investigation, I have observed in the A.V. bundle of the rat, dog and domestic fowl, strands of the same terminal network with associated nuclei as observed in the other parts of the ventricular wall. Akkeringa (1949) and Meyling (1953) have recorded a similar finding. Field (1951) who has investigated the nervous component of the A.V. bundle in a number of mammals, observed that individual endings are "conspicuously absent" in the A.V. bundle. Instead he reported the presence of a network of fine nerve fibres.

I could not, however, see this terminal nerve network in contact

with the peripheral Purkinje fibres, as was found by Meyling (1948) and Akkeringa (1949). although I have observed strands of network lying very close to them. Nonidez (1943), Stotler and McMahon (1947), Tcheng (1951) and Davies et al., (1952) also did not find nerve endings in relation to the peripheral Purkinje fibres. In the opinion of Stotler and McMahon, the terminal Purkinje fibres are entirely devoid of nerves but Davies et al., (1952), like me, found nerve fibres lying close to these cells but they were not seen to end in any particular relationship with them. Some of my sections, however, contained structures which at first sight gave the appearance of small bulbus endings of individual nerve fibre but on scrutiny the appearance of such endings was found to be due either to the cut ends of the fibres or to the sudden bending of the fibres.

As regards the innervation of the coronary arteries I have found, especially in the bird and mammal, rich perivascular plexuses, similar to those observed by others (Woollard, 1926; Stöhr, 1932; and Davies et al., 1952). However, it appears to me that the vascular innervation is limited to the adventitia and the subjacent part of the media. I could find no nervous structure in the deeper part of the media and in the intima, although Woollard (1926), described an enormously rich supply of the finest non-medullated nerve fibres in the muscular coat. Davies and Blair (1935) claimed to have seen intracytoplasmic nerve endings in the muscle cells of the media. Boeke (1932) observed that in the arteries single nerve endings could only rarely be found between the muscle cells of the media and he

took them to be artefacts due to vagaries of technique. Davies et al.,(1952) like Boeke could only occasionally find nerve fibres entering the media of the arteries. Field (1951) also could find no endings in the wall of the coronary arteries. The only types of ending I could find, and these were confined to the adventitia and the subjacent media, were thick medullated fibres which gradually became thinner and thinner and finally disappeared and the strands of the terminal nerve network. Similar thick tapering nerve fibres in the wall of the coronary arteries have also been described by Woollard (1926) and Davies et al.,(1952), although they could find no terminal nerve network. Instead they found small bulb-like nerve endings. Structures resembling such endings certainly occurred in my preparations but on close examination they proved to be artefacts. Woollard (1926) expressed the opinion that the capillaries are entirely without any nerve supply, while others (Davies and Blair, 1935, Nettleship, 1936, Field, 1951, Davies et al., 1952, Meyling, 1953) have described a rich capillary innervation. Many observers are convinced that the capillaries of the heart are accompanied by strands of the terminal nerve network (Meyling, 1953, Stöhr, 1957) and the present investigation has corroborated this finding.

It is interesting to note that certain invertebrates like the Coelenterate, have no organised central nervous system but have a network of nerve fibres with ganglia, which runs diffusely and almost uniformly throughout the body. Meyling (1953) and Mitchell (1956) have compared such a network in the Coelenterate with the terminal

nerve network found in the vegetatively innervated organs of the higher animals. This similarity between the terminal nerve network of the vertebrates and the primitive nervous system of the animals like Coelenterates suggests that it is very probable that the autonomic end-apparatus of the vertebrates has remained evolutionally in a relatively unchanged primitive state, although the rest of the nervous system, both somatic and autonomic, has undergone a process of gradual evolution reaching its highest development in the primates and especially in man.

SUMMARY

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(1) Intrinsic innervation of the cardiac ventricles of the representative classes of vertebrates were studied by Gros-Schultze method and Bodian's activated Protargol method. Three rat hearts were also studied with Nonidez's method.

(2) In the higher vertebrates, most of the ventricular nerves travel along the coronary arteries and their branches, whereas in the lower vertebrates most of the nerves reach the ventricle independently of the vessels.

(3) Larger nerves are arranged in a plexiform manner in all the three layers of the ventricular wall.

(4) Vertebrate ventricles are richly innervated but comparatively the avian ventricles have the richest nerve supply.

(5) Intrinsic autonomic ganglion cells are present in the ventricles of all animals examined except the fish. These nerve cells are situated mostly subepicardially, but a few are also present within the substance of the myocardium of the dog, rat, domestic fowl and frog. Intramural nerve cells were not encountered in the ventricles of lizard, grass snake and fish.

(6) The majority of the intrinsic ventricular nerve cells are multipolar in character. A few cells are probably unipolar or bipolar in form but their exact nature could not be ascertained with certainty.

(7) No organised discrete nerve endings are present in the ventricles. Instead a tridimensional terminal nerve network with associated cells is present in all the layers of the ventricular wall. Apart from the terminal nerve network, thick single medullated fibres of a terminal nature but without any organised endings are also present in all the layers of the ventricular wall.

(8) The bulbus cordis as a separate chamber, is present only in the frog amongst the animals investigated and has the same pattern of innervation as that of the ventricle.

(9) Specialised conducting tissue of the A.V. bundle is present only in the ventricles of the higher vertebrates viz. birds and mammals and is not found in the ventricle of the lower vertebrates.

(10) There is a species difference in the nervous component of the A.V. bundle of the animals in which the conducting system is present.

(11) Coronary innervation is confined to the tunica adventitia and the subjacent part of the media, in which the same terminal nerve network and single medullated fibre of a terminal nature are present. Capillaries are surrounded by strands of the same terminal nerve network.

(12) It is postulated that the autonomic end-formations in the vertebrate animals have evolutionally retained a primitive morphological character while the rest of the nervous system has undergone higher development.

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ADDENDUM

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Since completing this Thesis I have had the opportunity to consult Sir Russell Brock's recent work entitled "The Anatomy of Congenital Pulmonary Stenosis" (1957). In this work he briefly describes both the comparative anatomy and the normal muscular architecture of the bulbus cordis. He regards that the bulbus cordis is present in both Teleostian and Elasmobranch fish, whereas in this Thesis I have considered that the bulbus is not apparent in the adult Teleostian heart; and furthermore I have expressed the belief that it is replaced, both morphologically and functionally, by the bulbus arteriosus. The question of nomenclature of the chamber of the vertebrate heart, immediately cranial to the ventricle has been discussed in considerable detail in Part I of the Thesis.

In connection with the arrangement of the muscle fibres of the right ventricle, Sir Russell remarks that the superficial fibres consist of transverse loops which continue over both ventricles. In all avian and mammalian hearts, however, which were dissected for the present investigation, I have been unable to confirm this arrangement. My invariable finding was that the fibres of the superficial layers (superficial bulbospiral and superficial sinospiral) pass over both ventricles and are orientated longitudinally with a slight spiral inclination towards the left and not transversely as Sir Russell states. I have described that the fibres of these layers

pass from their origin at the base of the ventricles towards the apex of the heart where they converge and form the two horns of the vortex. This orientation, I considered, was adequately demonstrated in Fig.27 which is a photograph of the superficial muscle layers of the sheep heart, and shows the longitudinal disposition of the fibres of these layers. Fibres of the deeper layers (deep bulbospiral and deep sinospiral), however, are arranged rather transversely (Figs.30 and 31).

I have never been able to identify clearly the infundibulum as a well demarcated part of the right ventricle, as indicated by Sir Russell, in any of my dissection of the avian and mammalian ventricles. In all my specimens, I have found that the muscle fibres of both superficial and deeper layers are arranged in the same respective planes, both over the infundibulum and the right ventricle proper; and no line of demarcation between the two can be made out (Figs.27 and 30). In the interior of the ventricle, likewise, no particular arrangement of the muscle fibres can be identified which demarcates the infundibulum from the right ventricle proper, except that the inner surface of the former is smooth and that of the latter is trabeculated.

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